Chapter 2

EFFECT OF LOW AMPHETAMINE DOSES ON CARDIAC RESPONSES TO EMOTIONAL STRESS IN AGED RATS

C. Nyakas, B. Buwalda, P.G.M. Luiten and B. Bohus

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ABSTRACT

Effect of low amphetamine doses on cardiac responses to emotional stress in aged rats. In young Wistar rats conditioned emotional stress can be characterized by a learned bradycardiac response to an inescapable footshock. In aged rats this bradycardiac response is attenuated and accompanied by suppressed behavioral arousal in response to novelty. In the present study, cardiac responses to emotional stress and behavioral reactivity to a novel experience in an open field were tested in aged and young rats under the influence of a low dose of d-amphetamine (AMPH, 0.5 mg/kg i.p.). AMPH administration in 27-month old rats reinstated the bradycardiac response to emotional stress, while it failed to influence the resting heart rate in the home cage. Age-associated differences in open field ambulation, present in drug-free conditions, were antagonized by low doses of AMPH (0.25 - 1.0 mg/kg). It is concluded that enhanced arousal by aminergic stimulation with AMPH in the aged rat invoked cardiac and behavioral response patterns resembling those at younger ages.

INTRODUCTION

Previous studies in this laboratory on young male Wistar rats have shown that exposure to certain types of emotional stress - i.e. to expectancy of an unavoidable footshock - leads to a characteristic, bradycardiac heart rate response. (2,3,18,27). This deceleratory type of conditioned cardiac response diminishes with age; it is reduced over one year of age, and is practically absent in aged and senescent rats (27). The behavioral characteristics of aged rats in stress conditions like novelty-induced arousal also differ from that of young ones. Depending on the experimental circumstances it either shows a delayed appearance (5) or is reduced as compared to young age (16,34). Old rats respond to stress with a delay in the increment of brain noradrenergic activity as compared to young controls (19), and consistently show a reduced response of brainstem and forebrain noradrenergic systems shortly after stress (32,36). The absence of a vagally mediated cardiac response to emotional stress in aged rats is likely to be related to deficient neuronal processing in arousal mechanisms. There is growing evidence for a common neural network including catecholaminergic pathways mediating both arousal and vagally evoked bradycardiac responses (4,9,13,15,35,37). Thus, a delayed response of neurotransmitter systems supporting arousal, e.g. from the ascending catecholaminergic system (22,30,36), may be part of the mechanism underlying the altered stress response in aging. The view that aged rats are potentially capable to perform physiological responses well comparable to young animals is supported by the finding that movement disorders of aged rats can be reversed by central dopamine receptor stimulation (21). Consequently, we assumed that by increasing the level of arousal, a reinstatement or enhancement of the conditioned bradycardiac stress response may be obtained in aged rats.

Such an increased arousal level was achieved by application of the psychostimulant

amphetamine (AMPH).

AMPH in low doses is known to elicit behavioral activation and to enhance performance in learned tasks without provoking overt stereotyped behavioral movements (7,11,12,24). This effect is considered to be mainly mediated through dopaminergic and to a lesser degree through noradrenergic neurons (20,24,25,26).

Thus, the aim of the present experiments was first: to study the cardiac and behavioral response of aged Wistar rats to emotional stress under the influence of a low dose of AMPH. Two age groups were compared, 27- and 5-month old, representing the aged and young life conditions in rats, respectively. The emotional stress condition was generated in a step-through type passive avoidance learning situation. Fear of punishment was induced by exposing the rat to the compartment in which an unavoidable, painful footshock was given the previous day.

The second objective of this study was to establish the optimal dose of AMPH. The dose-dependent impact of AMPH administration was investigated on the response of the experimental animals in a novelty-induced arousal test in a small open-field.

The third question to answer dealt with the age-dependency of behavioral motor activation by AMPH in the open-field mobility test.

METHODS

Animals

The majority of the observations were carried out on two groups of male Wistar rats of 5 and 27 months of age. The age-dependency of the AMPH effects was studied in two additional age groups of 21 and 33 months. The animals originated from the Ivanova substrain and were kindly donated by Merck, Darmstadt, Germany. All rats were housed in groups of 4 or 5 animals in a light-controlled room (lights on from 7.00 to 19.00). After implantation of two subcutaneous stainless steel electrodes for electrocardiogram (ECG) recording (2), each rat was individually housed in a quadrangular plexiglass cage. Food and water were supplied ad libitum. All experiments were performed between 9.00 to 13.00 hours.

Dose- and age-dependency of AMPH effects

Dose-dependency of AMPH effects

An initial open-field behavior experiment was carried out to assess the dose-dependent behavioral effects of AMPH and to select an optimal dose for the subsequent experiments. The dose-dependent change of behavioral response to AMPH was compared in aged (27-month old) vs. young (5-month old) rats.

d-Amphetamine sulfate (OPG, Utrecht, The Netherlands) dissolved in physiological saline was injected i.p. in a dose range of 0.05, 0.25, and 1.0 mg/kg body weight. Controls were injected with the

saline solution. Drugs and saline were administered in an injection schedule of a random cross-over design to the same group of 7 rats per age. In this design drug and saline injections were alternated. Furthermore, a two days wash-out period was allowed between two injections after AMPH was given.

The AMPH effects were assessed by measuring novelty-induced behavioral arousal in a small open field (SOF). A rectangular box with transparant walls (20 x 20 x 30 cm; l x w x h) served as the SOF. Behavioral activities were scored every 15 sec during a period of 60 min, with a parallel observation of 8 rats. The following behavioral items were distinguished: rearing, walking, head turning with sniffing (ambulatory and exploratory types of movements here called locomotion for the sake of simplicity), face washing, licking of the body hair (elements of grooming), and immobility or sleep.

Age-dependency of AMPH effects

Based on the experience obtained in the previous experiment, the age-dependency of the AMPH effects in the open-field test were assessed by applying a single selected optimal dose of 0.5 mg/kg AMPH. The behavioral response to AMPH and saline treatment in the SOF was quantitatively analyzed in young and in two additional aged groups of 21 and 33 months.
In all SOF tests drugs or saline were administered 10 min before the start of the 1 h observation period.

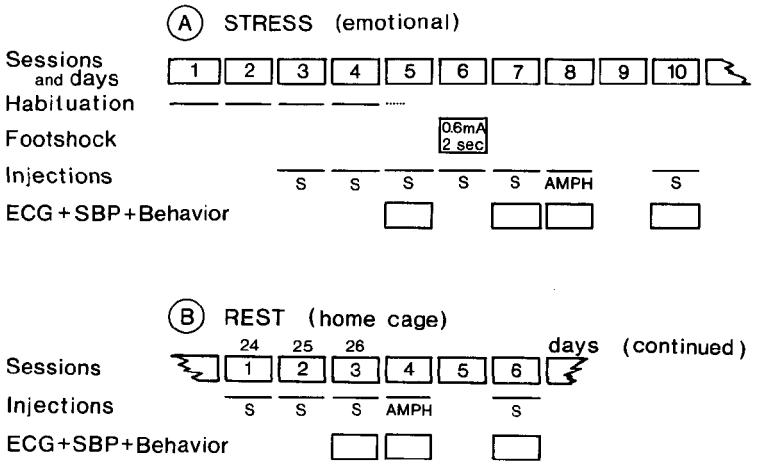

Fig.1. *Experimental protocol for sampling electrocardiograms (ECG), systolic blood pressure (SBP), and behavioral activity measurements in A) emotional stress (dark-compartment of a passive avoidance apparatus) and B) rest conditions. S: saline injection, AMPH: 0.5 mg/kg d-amphetamine sulfate.*

Heart rate and behavior in stress and rest conditions

Experimental procedure in the stress condition.

A one-trial learning paradigm in a step-through passive avoidance test (1) was used to investigate the cardiac and behavioral response to emotional stress. The experimental procedure started with habituation to experimental circumstances as well as to intraperitoneal injections with saline (see experimental protocol in Fig. 1). The experimental procedure included 1) fixing of a belt around the chest to hold a miniature radio transmitter (3 g), 2) carrying the rat from the home cage to the experimental room, and 3) placing the animal into a waiting cage for one minute. The waiting cage was located next to an experimental box designed for passive avoidance training. The passive avoidance set up consisted of a large black box or shock-compartment, in which an electric footshock could be delivered through the wiring of a grid floor. First the rat was placed onto a well-lit narrow platform attached to the dark shock-compartment. The rat could enter the dark through a small door to avoid intense light while staying on the platform. When the rat entered the dark-compartment, the sliding door was closed and the animal allowed to explore the dark chamber for 5 min. This procedure was repeated throughout 4 daily sessions. Beginning with the third session, 20 min before each transport to the experimental room, a saline injection was given intraperitoneally. At the 5th daily session, ECG measurements were started to obtain "preshock" values, i.e. control heart rate values prior to generating a one-trial learning fear reaction in the avoidance situation. ECG recordings started directly upon entrance in the dark-compartment and lasted for a period of 1 minute. On day 6 an inescapable electric footshock (0.6 mA, 2 sec) was delivered immediately after entering the dark-compartment. The rat was removed after 1 min and returned to the home cage. To obtain "postshock" ECG values, the rats were subjected to the experimental situation on days 7, 8, and 10. In all three daily sessions ECG's were recorded as described above. On day 8 rats was injected with AMPH in a dose of 0.5 mg/kg b.w., i.p., 20 min before transport to the experimental room. On days 7 and 10 the same animals were injected with saline.

During ECG measurements the duration of exploratory types of movements were recorded. These movements were: rearing, walking, and head movements with sniffing. The behavioral activity time was processed for data analysis.

Experimental procedure in the rest condition

Two weeks after the completion of studies in the stress condition, the resting heart rate and behavioral reactivity to AMPH were recorded in the home cage. The experimental procedure lasted for 6 days. Seven rats per group received a saline injection at sessions 1, 2, 3 and 6, 20 min before ECG and behavioral recordings. AMPH was injected i.p. in a dose of 0.5 mg/kg b.w. (i.p.) on session 4. The ECG and behavioral samplings of 1 min were carried out at sessions 3, 4, and 6. The behavioral activity was measured in seconds.

Recording and analysis of ECG

The electrocardiogram (ECG) of freely moving animals was monitored by means of a miniature FM transmitter (model SNR 102F, Dynamic Electronics Ltd, London, England) as described earlier (2).

Briefly, the transmitter was attached to a velcro strap secured around the thorax of the rat. The transmitter was connected to the two transcutaneous electrodes. The transmitted signals were received on a commercial FM receiver, amplified with half-amplitude cut-off frequencies at 10 and 100 Hz (Grass P5CR preamplifier) and stored on tape (Minilog, Philips).

For computer analysis the prerecorded ECG samples were played back through a cardiotachometer pulse generator, which generated a square wave pulse at each R wave. The original ECG recording and the generated R wave pulses were visualized on an storage oscilloscope for visual control. The time between the onset of two consecutive pulses, defined as interbeat interval (IBI) was measured by a personal computer (Olivetti M24) equipped with an interface. The IBIs falling within the range of 100 to 220 msec have been selected for computing the mean IBI of each sampled period.

Systolic blood pressure

Blood pressure was measured non-invasively either immediately after the ECG sampling in the emotional stress situation or after the one minute ECG and behavioral recordings in the home cage. The systolic blood pressure (SBP) measurement was carried out under light ether anesthesia with the tail-cuff method using a photoelectric sensor unit to detect arterial pulses visualized on an oscilloscope. The pressure in the cuff placed around the base of tail was gradually elevated till the disappearance of pulsation. During gradual decreasing of the pressure the SBP was red on a manometer at the moment of the reappearance of pulses and expressed in mmHg.

Statistical analysis

For the statistical evaluation of the data two-factor analysis of variance (ANOVA) with or without repeated measures was applied according to the STATS PC program. Post hoc pairwise comparisons between two groups was carried out by t-test corrected for repeated comparisons as appropriate. The paired t-test was used after ANOVA with repeated measurement on one factor.

RESULTS

Dose- and age-dependency of AMPH effects on novelty-induced behavioral activation

The dose-dependency of changes in behavioral activation after AMPH treatment were tested in a small open field. Both locomotion and grooming of 5- and 27-month old rats showed dose-dependent changes (Fig. 2). Two-factor ANOVA with repeated measures showed that the smallest dose used (0.05 mg/kg) already significantly increased locomotor activity ($F[1,12] = 5.01$, $p < 0.05$). The dose-dependent increase in locomotion was highly significant in both age groups ($p < 0.001$). The baseline difference between the two age groups (0.0 mg/kg, $t = 3.12$, $p < 0.01$, post hoc comparison with t-test) persisted at the lowest dose level

(0.05 mg/kg, t = 2.68, p < 0.02), but disappeared at higher doses (0.25 and 1.0 mg/kg). The suppression of grooming behavior by higher doses proved to be age-dependent and was present only in the young animals (significant effect of doses: F[3,18] = 8.93, p < 0.01, and age x doses interaction: F[3,36] = 3.69, p = 0.02). This experiment also served to select an optimal dose of 0.5 mg/kg AMPH for the cardiovascular and other behavioral studies. This dose was higher than the effective dose of 0.25 mg/kg but lower than the highest dose used (1.0 mg/kg), which evoked an exaggerated behavioral activation.

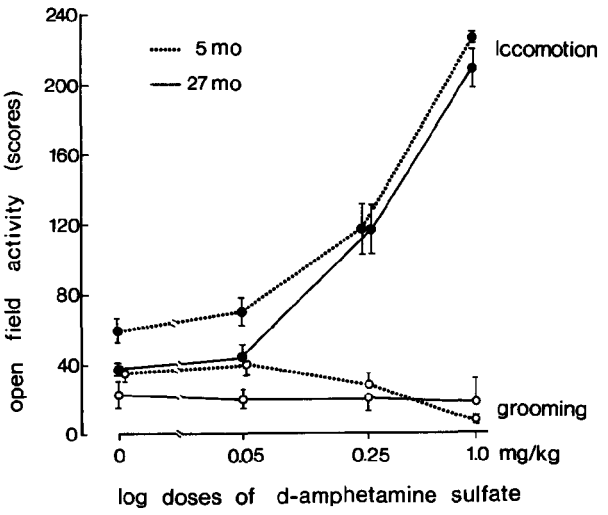

Fig. 2. Dose dependent effects of d-amphetamine sulfate on locomotor and grooming activities of 5- and 27-month old rats in the small open field test. Means ± SEMs are plotted from 7 animals per group. Locomotion scores are sums of rearing, walking, and head movement scores.

In a second behavioral experiment the age-dependency of AMPH effects on novelty-induced behavioral activity was studied in 5-, 21- and 33-month old rats, representing young, aged and senescent ages (Table 1). A single dose of 0.5 mg/kg AMPH or saline was injected. The frequency of occurrence of exploratory movements, i.e. rearing, walking and head movements as well as that of grooming and immobility was statistically handled by two-factor ANOVAs comparing the two treatments and the three independent age groups, respectively. AMPH markedly influenced the expression of all behavioral items (effect of treatment, p < 0.001) except grooming which was only slightly changed (p < 0.05). With respect to rearing and head movements a significant age x treatment interaction (p < 0.001) showed that young rats responded first of all with an increase in rearing activity, while senescent rats displayed more locomotor movements like head turnings and sniffing. Furthermore, AMPH treatment increased walking and decreased grooming without a significant age-dependency. Under drug-free conditions, the frequency of exploratory movement and grooming decreased, while immobility increased with age. Those behavioral parameters like walking and immobility, which were different in old (21-month) versus young animals (5-month), reached similar levels of appearance after AMPH. Immobility in senescent rats was greatly reduced by AMPH treatment but did not reach the same low levels as in adult rats.

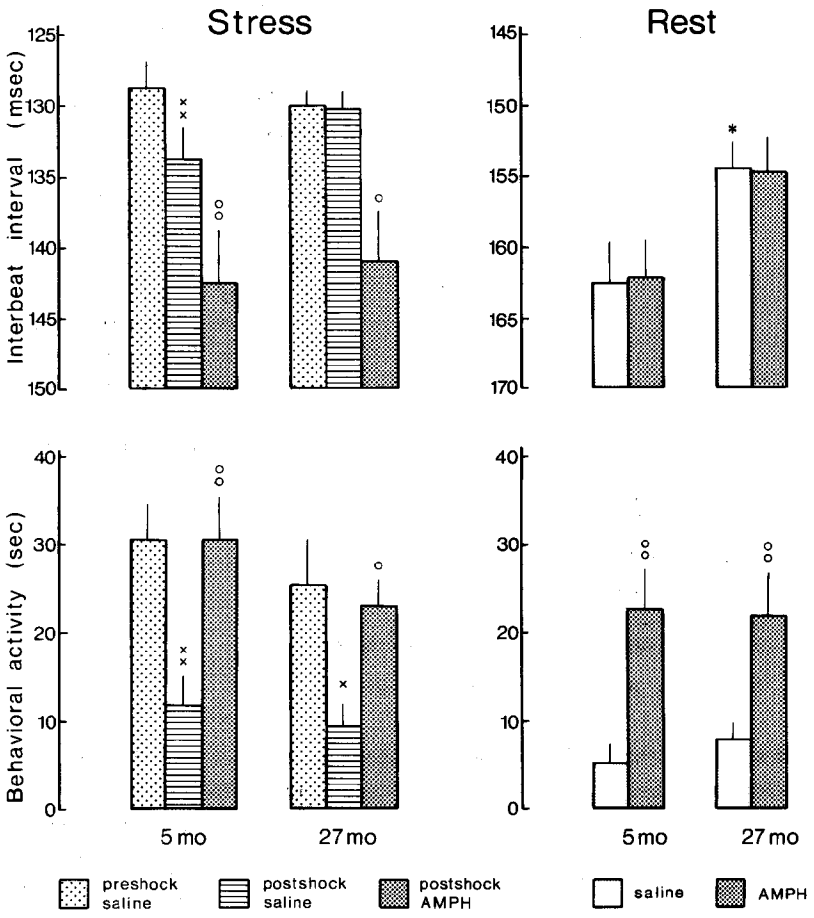

*Fig. 3. Cardiac and behavioral effects of low dose AMPH (0.5 mg/kg d-amphetamine sulfate) in young and aged rats. Heart rate expressed as IBI and behavioral activity are shown in stress and rest (home cage) conditions. Data are means ± SEMs from 7 animals per group. Columns represent the following sessions: under the heading "stress": preshock saline - day 5, postshock saline - averaged values from days 7 and 10, postshock AMPH - day 8; under the heading "rest": saline - averaged values from sessions 3 and 6, AMPH - session 4 (see also Fig. 1). Paired t-test was used to compare data sets of adjacent columns after completion of two-factor ANOVAs with repeated measures on sessions: xx $p < 0.01$ vs preshock saline control, o $p < 0.05$ and oo $p < 0.01$ vs postshock saline control, * $p < 0.05$ vs saline treated young rats in the home cage.*

Cardiac and behavioral effects of amphetamine in stress versus rest conditions

The heart rate and behavioral data obtained in the two experimental conditions, emotional stress and rest, are shown in Fig. 3. A two-factor analysis of variance with repeated measures on one factor, sessions, was applied for statistical evaluation. The heart rate expressed as interbeat intervals was not significantly influenced by the age factor in the stress condition. In the home cage aged rats had a higher heart rate in the drug-free condition ($t=2.27$, $p<0.05$, post hoc t-test). Analysis of variance on pre- and postshock saline values revealed that only the 5-month old animals responded to emotional stress with bradycardia (significant interaction between age and footshock, $F[1,12] = 18.9$, $p < 0.001$). The heart rate change in aged rats was negligible. In contrast, both age groups displayed a comparable immobility in the emotional stress state indicating a learned behavioral response (61 and 64 percent significant decrease in activity of 5- and 27-month old rats, respectively).

Treatment with 0.5 mg\kg AMPH induced a behavioral activation both in stress and in rest conditions. In the course of the postshock sessions a marked bradycardiac response appeared, $F[1,12] = 23.7$, $p < 0.001$, which was independent of age since no interaction between age and AMPH treatment was found. Young rats displayed an augmented bradycardia, while aged animals showed a clear bradycardiac response as compared to postshock saline control levels ($p < 0.01$, see Fig. 3). In the home cage, i.e. in the rest condition, although AMPH evoked behavioral activation it did not change heart rate values. The appearance of the bradycardiac response, therefore, was dependent on the experimental situation and only present in the stress condition.

Amphetamine and systolic blood pressure in stress and rest conditions

The effects of AMPH on SBP in stress and rest conditions are shown in Table 2. During stress there was no significant change in SBP across the postshock sessions in both age groups, although a slight SBP decrease after AMPH was observed. In rest conditions, however, there was an effect of AMPH, but only in aged rats. Two-factor ANOVA with repeated measures revealed a significant treatment effect in rest, $F[2,24] = 6.87$, $p < 0.005$, which was due to the hypotensive action of AMPH in the aged animals since a significant treatment x age interaction was also found ($F[2,24] = 3.87$; $p < 0.05$).

TABLE 1

Age-typical expression of amphetamine-induced behavioral activation in a small open-field test

Behavior	Treatment	Age in Months 5	21	33
Rearing	AMPH	51.5 ± 7.8*¶	10.5 ± 3.0+	5.6 ± 2.7+
	Saline	26.1 ± 2.5	5.9 ± 1.7+	1.6 ± 0.6+
Walking	AMPH	58.5 ± 6.2¶	62.1 ± 6.6¶	42.4 ± 8.1+¶
	Saline	22.6 ± 2.9	11.3 ± 3.3+	6.8 ± 1.3+
Head turn	AMPH	82.1 ± 7.4¶	88.8 ± 10.3¶	114.9 ± 8.6+¶
	Saline	47.5 ± 6.1	30.9 ± 4.5	15.6 ± 2.9+
Grooming	AMPH	6.9 ± 1.7§	17.5 ± 7.1	7.9 ± 2.2
	Saline	21.0 ± 3.0	26.1 ± 7.5	11.6 ± 1.5+
Immobility	AMPH	40.3 ± 6.3¶	65.0 ± 14.5¶	70.4 ± 15.2+¶
	Saline	123.1 ± 8.8	164.6 ± 9.7+	204.6 ± 6.7+

* Mean ± SEM scores.

Each group contained 8 animals.

AMPH (0.5 mg/kg) was given 10 prior to test.

+ $p < 0.05$, + $p < 0.01$ vs. 5-months-old rats (post hoc t-test)

§ $p < 0.05$, ¶ $p < 0.01$ vs. age-matched saline control (post hoc t-test)

TABLE 2

Effect of amphetamine on systolic blood pressure (SBP) of aged and young rats in stress and rest conditions

	SBP (mmHG) Saline +	AMPH +	Difference	p<
27 Months	139.4 ± 5.9*	132.4 ± 6.3	- 7.0 ± 3.2	n.s.
Stress				
Rest	142.6 ± 7.1	127.9 ± 6.7	-14.7 ± 5.9	0.05
5 Months	148.8 ± 3.5	146.3 ± 4.9	- 2.5 ± 5.6	n.s.
Stress				
Rest	142.8 ± 4.5	140.8 ± 5.0	- 1.6 ± 2.1	n.s.

* Mean ± SEM

+d-Amphetamine sulfate (0.5 mg/kg)

+ Mean of two saline sessions (7 and 10) preceding and following the drug session at day 8.

p < probability level (paired t-test)

DISCUSSION

Bradycardia is a typical cardiac response in young adult rats to conditioned stress situations evoking behavioral immobility. This decelerated heart rate response does not occur during aging. The main finding of the present experiments is that in aged rats treatment with a low dose of AMPH restores the emotional stress-induced bradycardiac response, while the drug does not influence heart rates in rest conditions. This indicates that a state-dependent effect of AMPH can be attributed to an emotional stress factor and not to a generalized pharmacological action of AMPH. The lack of a bradycardiac response to emotional stress in aged rats confirms our earlier observations (27). In contrast to the heart rate response, aged rats showed the same behavioral immobility response in the conditioned avoidance situation as do young controls. Therefore, the lack of bradycardya after conditioned avoidance learning in aged rats is not due to a memory deficit but to an autonomic dysregulation of heart frequency in response to emotional stress probably involving the vagus nerve (23,33). The baseline heart rate was higher in the aged rats suggesting a lower parasympathetic tone. The finding that the resting heart rate increases with age is consistent with some previous studies (23). Others, however, report no change (10) or a decreased heart rate in aged rats (6) indicating that this phenomenon may be strain dependent. In accordance with our current findings, studies in humans have also shown a reduced basal parasympathetic regulation of heart rhythmicity during senescence (29). In the presently used experimental design a relatively high heart rate could be observed in the dark-compartment of the avoidance set up, a situation in which both the aged and young non-stressed groups showed the same level of tachycardia. One may therefore conclude that the age-related suppression of parasympathetic cardiac response was not accompanied by an altered sympathetic unconditioned response in the aged rats.

The aged rats under drug-free conditions did not react to emotional stress with bradycardia, but performed the bradycardiac response at similar levels as young animals under the influence of AMPH. The AMPH treatment apparently reinstated the stress-induced vagal activation in aged rats and increased the amplitude of the response in young rats. Measuring locomotor activity during ECG recording in the stress situation after AMPH treatment clearly showed that both aged and young rats increased their activity to a comparable level. From a physiological viewpoint one would expect an increased heart rate of drug-treated rats as a result activation by somatic coupling (28). The present findings, however, indicate that cardiac rhythmicity is not automatically adjusted to somatic activity, but is apparently dominated by psychosomatic regulation processes in emotional stress conditions (8,27).

To obtain a more detailed picture of the autonomic response to AMPH in aged rats, the systolic blood pressure was also measured. The SBP was determined in relation to the heart rate values and measured immediately after the ECG sampling, i.e. approximately 25 min after the drug/saline injections. Amphetamine had no significant impact on SBP levels measured in stress conditions in either age groups. In the rest condition the 27-month

old rats showed a slightly lower SBP level when treated with AMPH. It is therefore unlikely that facilitation of the bradycardiac response of the AMPH treated rats did result from a simple baroreceptor reflex activation.

The behavioral immobility response in the dark-compartment the day after the footshock, was considered as a sign of successful learning and memory processes. In this respect the 27-month old rats performed similar as the younger counterparts. In our previous study (27) a deficit in passive avoidance learning could be observed only in senescent rats above the age of 30 months. Depending on the complexity of the task, the behavioral paradigm used, the experimental circumstances, but also on the exact age of experimental animals, aging rats may show deficits in their capacity for learning and memory (14,17,31,38). Both the presence and lack of cognitive deficits in aging animals should be viewed with appropriate criticism since motor or sensory dysfunctions might also be present and influence cognitive performance (14,31). The psychostimulant AMPH has been reported to facilitate learned behavioral responses not only in young but also in aged rats (11). Moreover, deficient motor coordination, which may hamper learned responses, could be antagonized with central dopamine receptor stimulation in aged rats (21). These latter studies and our present results with AMPH indicate that age-related decline in performance may for a considerable part be attributed to a deterioration in the central control mechanisms underlying cognitive and motor functions.

In general, the behavioral activation in response to novelty decreases with age (5,14,16,34), suggesting a deficient behavioral arousal. Some authors (5) observed a delay in behavioral arousal, while others described an age-related shift in the type of behavioral response to novelty (34). This is also apparent from age-related differences in the composition of movement patterns in response to novelty in the present study. A reduced behavioral arousal in aged rats is indicated by the observations on the 21-, or 27-month old animals showing a series of behavioral signs of reduced novelty-induced small open field (SOF) activity. This behavioral suppresssion was even more pronounced in the senescent 33-month old rats.

The behavioral activation due to AMPH treatment showed an age-typical appearance. Induction of rearing responses decreased with age, while aged rats performed more hypokinetic types of motor movements like head turning and sniffing. Amphetamine abolished the higher incidence of immobility of 21-month aged rats, and highly reduced immobility of the 33-month old senescent rats. Similarly, as in the other SOF experiment designed to study the dose-dependency, the decreased exploration seen in 27-month old rats was not observable when 0.25 mg/kg or higher AMPH doses were administered. Taken together, the considerable differences between the various age groups in the appearance of novelty-induced behavioral arousal was antagonized by low doses of AMPH. Amphetamine-treated aged rats were able to display an overall activity level equal to young controls. The behavior of senescent rats (33-month old) was also markedly influenced by AMPH, albeit without a full restoration of behavioral arousal. In conclusion, the age-related differences observed in adult versus 21- or 27-month old rats in both the cardiac and behavioral

responses were annihilated by low doses of AMPH.

As to the mechanism of the AMPH effect, the current data suggest that this psychostimulant drug increases stress-related arousal both in young and aged rats. AMPH reinstated the stress-evoked central neuronal drive of the cardiodeceleratory (vagal) response in aged rats to levels observed at young age. The reported effects of the drug, which appear to be state dependent, support the assumption that the neuronal processes underlying arousal play a permissive role for the emotional stress evoked bradycardia in the aged rats.

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Chapter 3

BEHAVIORAL AND CARDIAC RESPONSES TO MILD STRESS IN YOUNG AND AGED RATS: EFFECTS OF AMPHETAMINE AND VASOPRESSIN

B. Buwalda, J.M. Koolhaas and B. Bohus

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ABSTRACT

Young (3 mo old) male Wistar rats showed a relative decrease in heart rate to a sudden silence superimposed on low intensity background noise. This bradycardia was accompanied by immobility behavior. In 26 mo old rats the magnitude of the heart rate response was reduced while immobility behavior remained in the same order of magnitude as in young controls. In the aged rats a shift in autonomic regulation of heart rate in the direction of increased sympathetic influence was indicated by the results obtained by blocking the autonomic input with atropine methyl-nitrate (0.5 mg/kg) or atenolol (1 mg/kg) given subcutaneously (s.c.) 30 min prior to testing. Pretest (30 min) administration of amphetamine (0.5 mg/kg s.c.) reinstated bradycardiac response in aged rats to a level seen in young ones. Arginine-vasopressin (AVP, 10 μg/kg s.c.) administered 60 min before the experiment markedly facilitated the cardiac response in young animals but failed to restore cardiac responses in aged ones. The immobility behavior in the peptide-treated aged rats was also absent.

The present findings suggest that a diminished central aminergic drive in aged rats is causing a reduction of the parasympathetic cardiac response to stress of sudden silence. The results also indicate an age-related vasopressinergic modulation of behavioral and cardiac responses to mild stress.

INTRODUCTION

In aging animals and humans, stress-related cardiac dysrhythmias and hypertension may involve autonomic dysfunctions (1, 18, 23, 24). Previous research in this laboratory was focussed on the age-related changes in the autonomic responses due to stressors inducing behavioral immobility. During this passive way of coping, young male Wistar rats appeared to react predominantly with a cardio-inhibitory response (3, 4, 13). The initial bradycardiac response to a conditioned emotional stressor of fear of inescapable footshock was absent in aged rats (18). Since this bradycardia can be abolished by atropine (Buwalda, unpublished results), one may consider it as a primarily vagally mediated response. Accordingly an age-related attenuation of parasympathetic control of cardiac functioning during emotional stress situations was suggested (18).

The behavioral arousal induced by novelty stress is either delayed in onset in aged rats (5) or reduced in magnitude (26). Increasing the level of arousal by administration of the psychostimulant amphetamine (AMPH) appeared to reinstate the bradycardiac response to the conditioned stress of fear of inescapable footshock in aged rats (16). In a similar experimental design the effect of the neurohypophyseal hormone arginine-8-vasopressin (AVP) was investigated. Experiments in freely behaving rats in various stressful situations suggested that AVP serves as an important modulator of the vagally mediated cardiac response to conditioned emotional stressors (2, 13). Peripheral administration of this neuropeptide also did restore the bradycardiac response in aged rats (17).

The question was raised as to whether the age-related diminution of the

bradycardiac responses and its aminergic and peptidergic modulation can be generalized for both conditioned (predictable) and non-conditioned (unpredictable) stress stimuli. Bradycardia can also be elicited in a non-aversive, unconditioned situation. Orientation and attention towards stimulus changes is accompanied by a decrease in heart rate in the rat (12). Sudden silence superimposed on low intensity background noise is eliciting bradycardia and immediate behavioral arrest with orientational movements (13). Accordingly, the behavioral immobility is then a common factor of the unconditioned and conditioned stress response.

The aim of this paper was to analyse behavioral and cardiac responses to the mild stress of sudden silence in young and aged rats, with special emphasis on accelerative sympathetic and decelerative parasympathetic influences on heart rate. In addition, the modulating effect of AMPH and AVP on cardiac and behavioral responses to sudden silence in young and aged rats was investigated.

METHODS

Animals and housing

Male Wistar rats of 3-4 and 26 months of age were used. The animals originated from the Winkelman substrain and were kindly donated by Troponwerke, Cologne, Germany. They were housed 6 to a cage (40x60x15 cm), with food and water ad libitum, in a temperature controlled environment of 21 ± 2 °C; the lights were on from 07.30 to 19.30 hr. All experiments were performed between 9.00 to 13.00 hr.

Surgery

In order to record the electrocardiogram (ECG), transcutaneous stainless steel electrodes made of standard paperclips were implanted under light ether anesthesia. One was placed between the scapula and the other in the midback, according to the method described previously (4). At least three days were allowed for recovery before the start of the experiment.

Procedure

The rats' behavioral and cardiac responses to a sudden drop in background noise were measured in a rectangular clear Plexiglas cage (85x60x60 cm), which we will designate as an "open field" in this paper. This open field was located in an acoustically isolated experimental room. The floor was covered with wood shavings. A noise generator produced a constant "mixed" background noise (65 dB, 2-8 KHz) in the experimental room. This noise was on from the time of entering the experimental room. Upon entering a miniature FM-transmitter for the ECG recordings was fixed on the rat and subsequently the animal was placed in the open field for 5 min on Day 1. The time interval from entrance to exposure to the open field lasted about 1 min. On Day 2, the test day, the animals were exposed again to the open

field for 5 min. After the first 2 min the background noise was then switched off, leaving the animal in almost total silence for the final 3 min. Heart rate and behavior recordings were taken 3 times for periods (P) of 60 sec. Second min recordings (P1) were considered to be pre-stimulus measurements. Third (P2) and fifth min (P3) recordings were regarded as response measurements. As a behavioral measure the time spent on "immobility" was determined by an observer during the three periods. Immobility was defined as almost motionless scanning of the environment with only minor head movements.

Recording and analysis of the ECG

The ECG of freely moving rats was monitored telemetrically by means of a miniature FM transmitter (model SNR 102F, Dynamic Electronics Ltd., London, England) as described earlier (4). The transmitter was attached to a velcrose strap secured around the chest of the rat and connected to the transcutaneous electrodes. The transmitted signals were received on a commercial FM receiver, amplified (Narco Bio-System Inc. Mod. FM-1100-7) and stored on tape by a commercial tape recorder. During recording and analysis, the quality of the ECG signal was continuously monitored on an oscilloscope.

Recorded ECG samples were played back through a cardiotachometer pulsegenerator (Schmitt-trigger) that generated a square wave pulse at each R wave. The interbeat interval (IBI), which is the time between onset of the two consecutive pulses, was measured using a personal computer (Olivetti M24). The mean IBIs were computed for periods of 55 sec. IBIs shorter than 100 and longer than 220 msec were discarded because these were likely to be due to artifacts. For the analysis of recordings after AVP treatment, the upper limit was set on 250 msec because of the excessive bradycardia in some of the young animals.

Autonomic blockade

Substances

To examine the degree of vagal contributions to the cardiac responses cholinergic muscarinic receptors at peripheral level were blocked with atropine methyl-nitrate (0.5 mg/kg b.w.). The ß1-adrenergic antagonist atenolol (1 mg/kg b.w.) was used to block the sympathetic input to the heart. Atenolol was used because of its relative selectivity for cardiac ß1 receptors and its minimal central nervous system actions (8). Saline (SAL) injections served as vehicle. All substances were administered in a volume of 1 ml saline/kg b.w. and applied subcutaneously (s.c.) 30 min prior to testing.

Experimental design

Fourteen young and fifteen aged rats were devided in 2 groups. Rats in group I were tested for their cardiac responses to stress of sudden silence after SAL administration. Three days later the animals were injected with atropine methyl-nitrate and tested again. Rats in group II were tested for cardiac responses to placement in the open field only in order to test the sympathetic activation. The animals in group II were injected with SAL followed 3 days later by atenolol.

Aminergic and peptidergic modulation

Substances

d-Amphetamine sulfate (AMPH, OPG, Utrecht, The Netherlands) was administered s.c. (0.5 mg/kg b.w.) 30 min prior to testing. Arginine-vasopressin (AVP) was injected s.c. (10 μg/kg b.w.) 60 min prior to the exposure to the open field. SAL was used as a vehicle. The substances were applied in a volume of 1 ml saline/kg b.w. The selection of the single doses of the peptide and the drug was based on the results of the previous studies of young and aged rats' cardiac response to the conditioned stress paradigm (16,17). Nyakas et al (16) showed that an AMPH dose of 0.5 mg/kg significantly enhanced behavioral activity of young and aged rats in a small open field to a same level without inducing stereotypic behavior. The AVP dose of 10 μg/kg was the most potent dose to reinstate the bradycardia in aged rats in the conditioned stress paradigm (17).

Experimental design

Nineteen young and eighteen aged rats were devided in two groups (groups III and IV). Rats in these groups were tested for their cardiac and behavioral responses to stress of sudden silence. Animals in group III were injected in a cross-over design with SAL and AMPH, each animal serving as its own control. Rats in group IV were injected in a cross-over design with SAL and AVP. Between administrations were 7 days to minimize interaction of treatments.

Statistics

Results are presented as means ± SEM. Cardiac data were analyzed using a multivariate analysis of variance with repeated measures (MANOVA-STATS program), a two-tailed Student's t-test and a paired t-test. Behavioral data were evaluated for significance using the Mann-Whitney U-test and the Wilcoxon matched-pairs ranked-signs test. A probability level of $p<0.05$ was taken as statistical significance for all tests.

RESULTS

Autonomic contributions to cardiac and behavioral stress responses

Fig.1A shows young and aged rats' cardiac responses to stress of sudden silence. MANOVA revealed a significant effect of age: lower heart rate was observed during all 3 periods in aged rats, $F(1,13)=11.61$, $p<0.01$. The interaction between age and periods was almost significant, $F(2,26)=3.07$, $p=0.06$. Young rats responded to sudden silence with a significant increase in IBI, i.e. a decrease in heart rate, ($p<0.01$). Aged rats also showed a bradycardiac response to switching off the noise ($p<0.01$). The magnitude of the bradycardia in aged rats, however, was smaller than the one seen in young ones ($p<0.05$).

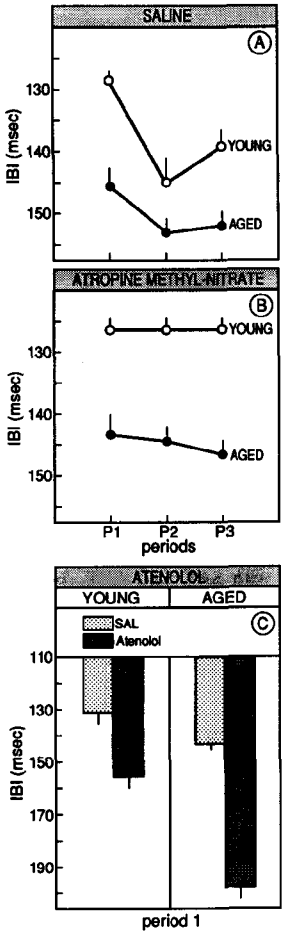

Fig.1

Heart rate expressed as interbeat interval (IBI) during a 5 min exposure to an open field before and after sudden reduction of non-aversive background "mixed" noise, measured in young (3 mo old) o-o and aged (26 mo old) •-• male Wistar rats. One min recordings were made during the second min of exposure to the field with noise on (P1); during the third min immediately after the noise was switched off (P2) and during the fifth min (P5). Young and aged rats' IBIs are presented after subcutaneous (s.c) administration of 0.5 ml saline [A] or atropine methyl-nitrate (0.5 mg/kg b.w.) [B] 30 min prior to open field exposure. The lower panel [C] shows IBIs only during the first recording in the open field (P1) 30 min after intraperitoneal (i.p.) administration of saline or atenolol (1 mg/kg b.w.). Means ± s.e. from 7-11 rats per group are shown.

Administration of atropine methyl-nitrate blocked the bradycardiac response to sudden silence in both age groups (Fig.1B). Heart rate after handling, transport and placement in the open field (P1), however, was not affected by atropine as revealed by a comparison with the P1 values of the SAL treated controls (Fig. 1A). The effect of age as observed in controls (Fig. 1A) was also preserved: lower heart rate appeared in the aged animals, $F(1,12)=26.32$, $p<0.001$). Fig.1C shows the mean heart rate values after handling, transport and placement in the open field (P1) in rats receiving atenolol. The difference in IBI between young and aged saline treated groups ($p<0.05$) was the same as in the former experiments (Figs.1A and 1B). Atenolol administration significantly increased IBIs both in young ($p<0.01$) and aged rats ($p<0.001$). The difference in mean IBI between the two age groups after atenolol treatment, however, was larger (43.6 ± 5.7 msec) than after saline

administration (11.4 ± 5.5 msec) ($p<0.001$).

Young as well as aged rats responded with a significant increase in time spent on immobility behavior (resp. 25 ± 7 sec and 24 ± 9 sec) ($p<0.05$). Administration of atropine and atenolol did not affect the behavioral responses (data are not shown).

Modulation of cardiac and behavioral stress responses by amphetamine and vasopressine

Amphetamine

The mean heart rate of young and old animals following vehicle injection was the same as in the former experiment and both young ($p<0.001$) and aged rats ($p<0.05$) responded to sudden silence with a bradycardia. The response in young rats was again significantly larger than the one seen in aged animals ($p<0.01$). AMPH administration did not cause a general treatment effect on IBI in young and aged rats. In the aged group, however, it did result in an interaction between treatment and periods, $F(2,40)=4.8$, $p<0.01$ (see table 1). Fig.2 and table 1 show that an increased bradycardiac response to sudden silence occurred in aged rats after administration of AMPH. The magnitude of the response was the same as in young animals. The cardiac responses to sudden silence in young rats were not affected by AMPH. Fig.3 shows that both young and aged rats treated with SAL responded behaviorally with similar increases in immobility ($p<0.01$). AMPH failed to influence significantly behavioral reactivity in young and aged rats, but increased the difference that was present between vehicle injected groups ($p<0.001$).

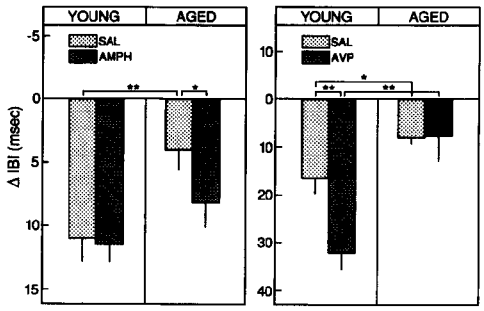

Fig.2

*Effect of Amph (0.5 mg/kg b.w.; s.c. 30 min prior to testing) and AVP (10 μg/kg b.w.; s.c. 60 min prior to testing) on heart rate responses in IBI (P2-P1) to sudden silence in young (3-4 mo old) and aged (26 mo old) rats. Means ± s.e. from 7-10 animals per group are shown. *P<0.05, **P<0.01 young vs aged (two-tailed t-test).*

TABLE 1

Effect of amphetamine on behavioral and cardiac responses to stress of sudden silence.

	3 Months (n=11)		25 Months (n=11)	
	IBI msec	immobility sec	IBI msec	immobility sec
Saline				
period 1	127 ± 2*	15 ± 4	143 ± 3	20 ± 4
period 2	138 ± 2	55 ± 3	147 ± 4	48 ± 6
period 3	131 ± 2	39 ± 8	147 ± 3	37 ± 7
Amphetamine				
period 1	128 ± 2	13 ± 3	142 ± 2	25 ± 6
period 2	139 ± 2	53 ± 3	150 ± 3	43 ± 6
period 3	137 ± 3	40 ± 4	154 ± 3	27 ± 6

** Mean ± SEM*

Cardiac rate, expressed as interbeat interval (IBI) in msec, in saline or amphetamine treated young and old rats tested in an open field. Duration of immobility during each period is given in sec. Period 1 represents a 60 sec recording just before the sudden reduction of background noise. Period 2 and 3 were recorded respectively immediately and two min after sudden silence.

TABLE 2

Effect of vasopressin on behavioral and cardiac responses to stress of sudden silence.

	3 Months (n=8)		25 Months (n=7)	
	IBI msec	immobility sec	IBI msec	immobility sec
Saline				
period 1	129 ± 2*	27 ± 5	145 ± 3	30 ± 6
period 2	147 ± 4	53 ± 6	153 ± 2	53 ± 3
period 3	139 ± 3	36 ± 8	152 ± 3	36 ± 5
Vasopressin				
period 1	168 ± 8	19 ± 5	172 ± 9	41 ± 5
period 2	201 ± 7	60 ± 0	179 ± 6	44 ± 8
period 3	197 ± 8	43 ± 6	176 ± 9	32 ± 10

** Mean ± SEM*

Cardiac rate and immobility behavior in saline and vasopressin treated young and old rats in an open field. For further information see Table 1.

Arginine-vasopressin

Table 2 shows that administration of AVP resulted in a general increase in mean IBI when compared to SAL treatment in both young, $F(1,14)=39.95$, $p<0.0001$, and aged rats, $F(1.12)=10.25$, $p<0.01$. AVP also caused a significant interaction between treatment and periods in young, $F(2,28)=8.5$, $p<0.005$, but not in aged rats (table 2). Like in the former experiments, SAL treated animals showed a significant bradycardiac response after sudden silence (Fig.2) with an age-dependent difference in the magnitude of the response ($p<0.05$). After AVP administration cardiac inhibition to sudden silence in young rats was much stronger when compared to SAL treatment ($p<0.01$). In aged rats AVP failed to enhance the magnitude of the bradycardiac response. The behavioral effect of AVP was also differential. An increase in immobility was observed in young rats, while an almost significant decrease in immobility response was found in aged rats ($p<0.06$).

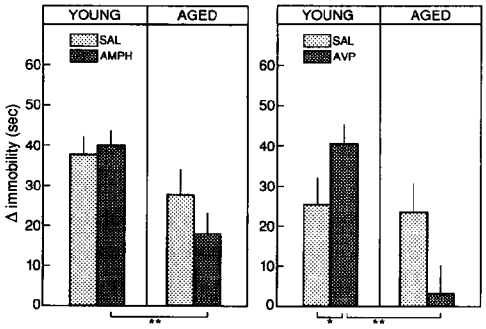

Fig.3

*Effect of Amph and AVP administration on behavioral responses to sudden silence in young and aged rats, measured in increase in time spent on immobility behavior (P2-P1). *P<0.05, **P<0.01 young vs aged (two-tailed t-test). For further information see Fig.2*

DISCUSSION

In the presented experiments the occurrence of sudden silence was used as a model for mild unexpected stress. The results show that this mild stressor caused a marked bradycardia in young rats. This bradycardiac response was diminished in aged rats. The results after blockade of parasympathetic or sympathetic input to the heart indicated a shift in autonomic regulation of the heart rate in aged rats. Finally it was demonstrated that AMPH, and not AVP administration increased bradycardiac responses in the aged animals to a level that was seen in young ones.

The diminished bradycardiac response to mild unexpected stress in aged rats confirms the earlier observations on the effects of an aversive, conditioned emotional

stressor (18). Since aged and young rats showed similar behavioral reactions to sudden silence, the lower bradycardiac response in aged rats is not attributed to a behavioral deficit but to an autonomic dysregulation of the heart in response to stress. The results after atropine administration indicate that the reduced bradycardiac response to sudden silence in aged rats is caused by a reduction of stress-induced parasympathetic responsivity in old animals. In accordance with this finding age-related decreases in parasympathetically mediated cardiovascular responses were reported in the magnitude of the baroreceptor reflex-induced bradycardia (22) and reduced heart rate variability (7).

Overall heart rate after transport, handling and exposure to the open field was lower in the saline treated aged rats when compared to young ones. This age difference was similar to the one found during resting conditions in another study (6), suggesting an age-related change in autonomic tone. Muscarinic blockade failed to affect the difference in heart rate. Since administration of atropine neither caused a shift in general heart rate towards acceleration during P1 in young or aged rats, tonic parasympathetic activation of the heart appears to be absent during this period. The lower heart rate in old animals is therefore not caused by an elevated parasympathetic activation. Blocking the ß1-adrenoceptors on the heart with atenolol increased the existing age-related difference in the heart rate of free moving rats. Accordingly, the lower heart rate cannot be ascribed to a reduced sympathetic drive in aged rats. Since both ß-adrenergic and muscarinic blockade fail to diminish the existing difference in heart rate, possibly a decreased intrinsic cardiac rate in aged rats is involved (19). Although it is generally accepted that ß-adrenergic stimulation of rat cardiac contraction is reduced with increasing age (21,25), the increased difference seen in heart rate between young and aged rats after ß-blockade seems to reflect an increased accelerative impact of the existing sympathetic tone on cardiac rate in aged Wistar rats.

AMPH administration, like in a conditioned emotional stress paradigm (16), restored the bradycardiac response to mild unexpected stress in aged rats to levels seen at young age. The normalized cardiac responses in aged rats cannot be ascribed to an improvement of behavioral responses: immobility behavior was even diminished in aged rats after AMPH treatment. Similar attenuation of immobility was observed in the conditioned stress paradigm (16). These findings suggest a discoupling of the organization of behavioral and cardiac stress responses. Since AMPH caused a slight decrease in systolic blood pressure 30 min after administration in both resting and stress conditions (16), it is unlikely that the facilitation of the bradycardiac response in aged rats did result from a simple baroreflex activation. AMPH administration did not influence cardiac and behavioral responses to stress in young rats. This probably indicates that the central drive of these particular responses in the SAL treated young group were already maximal under these stress conditions. It is also possible that the sensitivity to AMPH is lower in the young animals, but our former dose-response study in a conditioned stress paradigm (16) does not support such a view. The drug action on cardiac response is probably due to an increased parasympathetic activation in aged rats. Atropine treatment appeared to abolish the cardiac effect of AMPH in old animals during conditioned aversive stress situations (Buwalda, in prep). Since AMPH

facilitates the release of biogenic amines through presynaptic mechanisms (14), a direct peripheral effect on heart rate would be of an acceleratory nature. Therefore, AMPH probably modulates autonomic stress responses through central mechanisms. This action is not simply due to aminergic activation in the brain, since AMPH failed to induce comparable effects in resting conditions (16). Rather, a modulation of stress-induced arousal is likely. The nature of the mechanism is now under study.

AVP caused a reduction in heart rate both in young and in aged rats. Nyakas et al (17) reported a moderate bradycardia in resting conditions and a slightly decreased mean blood pressure 60 min after the subcutaneous administration of 10 μg/kg AVP in both resting and stress conditions. It is therefore unlikely that the general reduction in heart rate as observed in the present experiment is the result of baroreceptor reflex-induced vagal activation. Vasopressin-induced enhancement of cardiac responses to unexpected stress was very strong in young while absent in aged rats. Furthermore the behavioral response to sudden silence was absent in aged rats after AVP administration. The responses seen in the young rats confirm earlier observations seen in mild stress (13). During conditioned stress situations bradycardiac responses in aged rats were enhanced by AVP (17). The aged rats used in that experiment, however, were 14 months old. The age difference in the two experiments may explain the lack of peptidergic reinstatement of the bradycardia in the present experiments. To what extent central and peripheral mechanisms are involved in the modulating properties of peripherally administered AVP on stress-related behavior and autonomic functioning is still a major issue of discussion (9,10,15). A decline in brain AVP innervation in aged rats (11,20) was reported, but data on central receptor states are not yet available. It is possible that the effects of AVP on behavioral and cardiac responses are caused by viscerally induced signals (15). To elucidate the exact mechanisms of action of peripherally applied AVP further investigation is needed.

In conclusion the present study provides evidence for a general decrease in parasympathetic cardiac inhibition to stressors evoking immobility behavior in aged rats. The results also indicate that vasopressinergic and central aminergic systems are involved in the stress-related parasympathetic drive.

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Chapter 4

REDUCED PREABSORPTIVE INSULIN RESPONSE IN AGED RATS: DIFFERENTIAL EFFECTS OF AMPHETAMINE AND ARGININE-VASOPRESSIN

B. Buwalda, J.H. Strubbe, M.W.N. Hoes and B. Bohus

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ABSTRACT

The experiments presented here have been designed to investigate whether the age-related attenuation of the vagal reactivity to emotional stressors and its modulation by amphetamine (Amph) or arginine-vasopressin (AVP) can be generalized for other physiological response patterns. We therefore studied the vagal control of the endocrine pancreas during food intake. Young (3 mo old) and aged (27 mo old) male Wistar rats were provided with permanent cardiac catheters allowing free movement and repeated, stress-free blood sampling. The vagally mediated preabsorptive insulin response (PIR) in relation to food intake as seen in young rats was reduced in aged ones. Blood glucose increments were the same at both ages. Administration of Amph (0.5 mg/kg; s.c.) 30 min before or AVP (10 μg/kg; s.c.) 60 min before presentation of a test meal lead to an elevation of the magnitude of insulin secretion in young rats but reduced the response in aged rats. Moreover, the PIR was not reinstated in aged rats. Blood glucose increments were not influenced by the treatments. The results are interpreted in terms of age-related general reduction of parasympathetic reactivity. The differential effect of amphetamine and AVP treatment on the insulin response suggests that the central aminergic or peptidergic drive of vagal output to the endocrine pancreas is also age-related.

INTRODUCTION

Physiological and neuroendocrine stress responses are markedly affected by age in animals and man (5,18,19,21,22). Previous studies in our laboratory have shown an age-related reduction in the initial bradycardia to a conditioned emotional stress (fear of inescapable footshock) (19). Since this response could be blocked completely by atropine (Buwalda, unpublished results) it is probably vagally mediated and a diminution of stress-related parasympathetic control of cardiac functioning during aging was suggested (19). Administration of the psychostimulant amphetamine (AMPH) appeared to reinstate the bradycardia response in aged rats (18). In a similar experimental design the effect of arginine-8-vasopressin (AVP) was investigated. AVP appeared to intensify the bradycardia response in young adult rats (3) and this neuropeptide restored the bradycardia in 14 months old rats also (18).

The question was raised as to whether the age-related diminution of vagal reactivity and its aminergic or peptidergic modulation can be generalized for other physiological response patterns.

During feeding plasma insulin level increases in the first minute of food presentation before an elevation in peripheral glucose can be observed (2, 24). It has been repeatedly demonstrated that this preabsorptive insulin response (PIR) is of cephalic origin (2, 8, 20,

25), and can be abolished by vagotomy (11, 12, 26) or atropine methyl-nitrate (2, 25). All of these results were obtained in Wistar rats, and although we do not show again that vagal activation provokes a PIR, it is reasonable to assume that the insulin response in these animals is mediated by the vagus nerve.

The present experiments were designed to investigate the pattern of the cephalic insulin secretion in young and aged Wistar rats during the presentation of the non-aversive physiological stimulus of food. In addition, the modulating effects of AMPH and AVP on this response were studied.

METHODS

Animals

Young (3 mo old) and aged (28 mo old) male Wistar rats were kept individually in Perspex cages (25x25x30 cm) at a room temperature of 20 ± 2°C and had continuous access to standard carbohydrate-rich food (Hope Farms Lab chow) and water unless otherwise stated. At the beginning of the experiments the young rats (n=8) weighed 289 ± 2 g and the aged rats (n= 6) 417 ± 9 g. They were housed on a 12 h light-dark cycle (the lights were on from 0.00 to 12.00 h.)

Experimental procedure

The rats were trained to consume a test meal consisting of 2 g of ground rat chow mixed with 2 ml water offered in a porcelain dish. Before the onset of the experiments the animals were food-deprived for 4 h between 9.00 and 13.00 h -i.e. in the period of high spontaneous food intake. The animals started to eat immediately after food presentation. Only those rats that consumed the test meal within 4 min participated in the experiments. The tests were performed between 13.00 and 16.00 h on separate days with an interval of at least 3 days. Young and aged rats were tested in randomized order to prevent differences in fasting period. The first experiment consisted of the presentation of a test meal to determine whether the PIR was reduced in the aged animals when compared to the young ones. In the second experiment the effect of treatment with d-amphetamine sulphate (AMPH, OPG, Utrecht, The Netherlands) or AVP on plasma insulin and blood glucose levels after a test meal was compared with that of a saline treatment. Animals were subcutaneously (s.c.) injected with saline 30 min before meal presentation. Three days later AMPH was injected s.c. in a dose of 0,5 mg/kg b.w. dissolved in saline (0.5 mg AMPH/ml) 30 min before the test meal. In a further test, AVP was administered s.c. in a dose of 10 μg/kg dissolved in saline (10 μg AVP/ml) 60 min before the presentation of the meal. The selection of these doses of AMPH and AVP was based on the results of the previous studies of young and aged rats' vagally mediated cardiac response to emotional stress (18).

Blood sampling

The rats were provided with a permanent catheter in the right atrium inserted via the right jugular vein and externalized on the head of the rat. This catheter allows repeated blood sampling in the

unrestrained and undisturbed rat (23). Surgery was performed under complete ether anesthesia. Before the start of the experiments the animals were handled for at least a week to accustom them to the sampling procedure. Blood samples of 0.25 ml were taken in vials containing 5 μl heparin (500 IU). In order to obtain relatively stable basal blood insulin and glucose levels, the meal tests were performed after a fasting period of 4 h. Blood samples were taken at 0 (start of meal ingestion),1,2,3 and 5 min. Withdrawn blood was replaced by transfusion of heparinized blood (25 IU per ml) of a donor rat fed ad libitum in order to minimize the changes in blood volume. A transfusion of 0.5 ml blood was given at -5 and between 3 and 5 min. After the fifth min sample a last transfusion of 0.25 ml was given.

Insulin and glucose determinations

Blood samples were immediately chilled and blood glucose was measured from whole blood by a ferrycyanide method with a Technicon analyzer. The remaining blood was centrifuged at 4°C. The plasma was stored at -20°C until analysis. Plasma insulin was measured by radioimmunoassay (NOVO-Denmark) using rat insulin as a standard, ^{125}I-labelled porcine insulin and antiporcine insulin guinea pig serum M 8309. Samples (25 μl) were measured in duplicate. Bound and free ^{125}I-labelled insulin were separated by polyethylene glycol (23.75% w/w in water) precipitation. The coefficient of variation of the immunoassay was < 8%.

Statistical analysis

The data were expressed as mean delta increases to basal levels at t=0 min ± standard errors of the mean (SEM). Statistical analysis was performed by using a Multivariate Analysis of Variance (MANOVA) followed by post-hoc t-tests. The paired t-test was used for comparisons within individuals. The criterion of significance was set at $P \leq 0.05$.

RESULTS

Table I shows the mean basal insulin and glucose levels in young and aged rats. No difference was found between untreated rats. There was also no significant change of basal levels following administrations of saline, AMPH or AVP.

Figure 1 shows the changes in plasma insulin and blood glucose levels in response to a test meal in young and aged rats. A significant rise in plasma insulin was observed in the first minute after the start of the meal in the young rats ($p<0.01$) with a progressive rise in the subsequent minutes. In the aged rats the early insulin increment was absent. During the following minutes a slower and smaller rise occurred in the plasma insulin levels when compared to young rats. The meal induced rise in plasma insulin reached significance only in the fifth min. Blood glucose levels rose sharply after the 2nd min of the test meal in both young and aged rats.

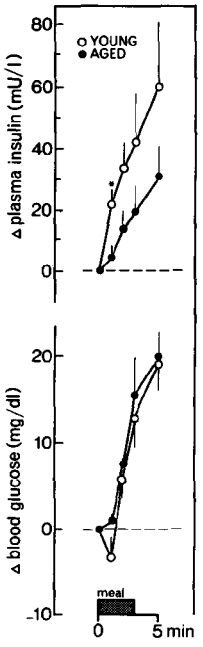

Fig.1

*Plasma insulin and blood glucose responses to a test meal in young (3 mo old) o-o and aged (27 mo old) •-• male Wistar rats. Means ± s.e. are from 6-8 rats per group. *P<0.05 young vs aged group (two-tailed t-test). The time 0 min represents the onset of the meal consumption (shaded area).*

TABLE 1

Basal levels of plasma insulin and blood glucose in young and aged rats

Treatment	Insulin μU/ml plasma		Glucose mg/dl blood	
	young	aged	young	aged
None	54 ± 6*	69 ± 5.4	95 ± 1.9	88 ± 2.8
Saline	67 ± 4.7	63 ± 2.7	96 ± 2.1	95 ± 2.5
Amph	66 ± 4.2	64 ± 4.8	93 ± 2.1	97 ± 4.1
AVP	69 ± 4.7	68 ± 7.4	98 ± 3.1	98 ± 3.1

* Mean ± SEM

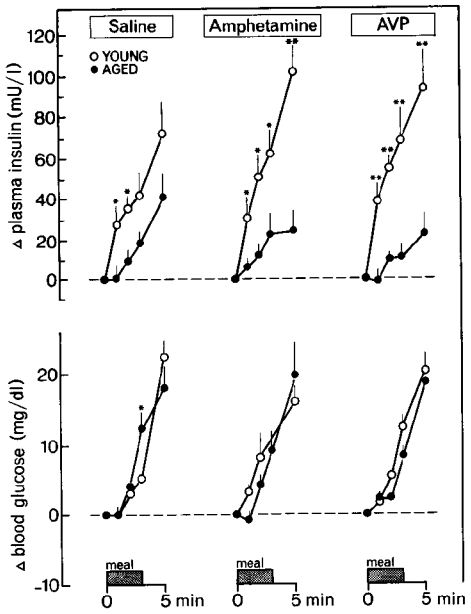

Fig.2

*Plasma insulin and blood glucose responses to a test meal in young (3 mo old) o-o and aged (27 mo old) •-• male Wistar rats after subcutaneous treatment with saline, amphetamine (0.5 mg/kg) or AVP (10 μg/kg) 30, respectively 60 min before onset of the meal. Means ± s.e. are from 5-6 rats per group. *P<0.05 **P<0.01 young vs aged group (two-tailed t-test). The time 0 min represents the onset of the meal consumption (shaded area).*

Fig 2 shows plasma insulin and blood glucose changes to the test meal after treatment with saline, AMPH or AVP. Under all conditions a significant rise in insulin levels was observed in the first min in the young rats ($p<0.05$). Differences between young and aged rats in the shape of the response curves could be seen after the second min of the meal. In young animals higher insulin values were found following amphetamine and AVP treatment. In contrast, insulin release to the test meal in aged rats was reduced and showed a significant increment only on the third min. Administration of the drug or peptide enhanced, albeit not significantly, the magnitude of the insulin response in young animals but slightly suppressed the response in aged rats. This differential effect of AMPH and AVP on insulin responses in both groups is indicated by the MANOVA with repeated measurements for the time points after start of the meal. There was no interaction between age and sampling time after saline treatment ($F(3,36)=0.22$, $p=0.88$), indicating that besides the difference in insulin secretion in the first min, insulin levels increased in a similar way in young and aged rats. This interaction, however, became highly significant after treating young and aged rats with AMPH ($F(3,24)=5.86$, $p=0.004$). Pretreatment with AVP had a similar effect ($F(3,27)=2.71$, $p=0.06$). Post-hoc t-test revealed that these interactions were caused by

a difference in the magnitude of the insulin response between young and aged rats following treatment with AMPH or AVP.
Premeal-meal differences in blood glucose levels never reached significance before the second min after presentation of the meal. As regards the glucose responses, MANOVA did not indicate a difference between the young and old animals after each of the treatments.

DISCUSSION

The first major finding of these experiments is a reduced preabsorptive insulin response in aged in comparison to young rats. One of the possible explanations is a diminished activation of vagal output during ingestion of a meal in aged rats. Alternatively, the pancreatic B-cell response to vagal activation may be reduced due to changes in cholinergic or other receptors. The reduced PIR in aged rats did not result in different glucose increments compared with the young during the first five min after start of the meal. The absence of alterations in blood glucose levels in the aged rats with diminished insulin response is surprising. Beside the impaired insulin response one would also expect changes in glucose response on the basis of decreased glucose tolerance during aging (6,9). However, studies with subdiaphragmatic vagotomized rats suggest that the early phase of the glucose response is not affected by this vagal section (26). The possibility remains open whether the impairment is revealed after a longer delay - i.e. beyond the measurements performed in the present investigation.

Age-related decreases in parasympathetically mediated cardiovascular responses were found in the magnitude of the baroreceptor reflex-induced bradycardia (21), the diminution of bradycardia resulting from an emotional stress in rat (19) and reduced heart rate variability in man (5). The present results may be viewed as an extension of this; aging resulting in a reduction of a vagally mediated response in the rat also to an unconditioned, non-aversive physiological stimulus like eating a small meal.

The results of the second experiment indicate that there is a differential effect of AMPH and AVP on insulin secretion in young and aged rats. In the young animals both substances affected mainly the magnitude of insulin secretion. In aged rats the peak insulin secretion is blunted and neither AMPH nor AVP treatment reinstated the PIR.

The major effect of AMPH is to facilitate the release of biogenic amines through presynaptic mechanisms (15). A direct peripheral effect of the drug on insulin secretion would then be expected to be of an inhibitory nature (1,4). It is therefore probable that the potentiation of the PIR in young rats is caused by enhancement of vagal drive by an action within the central nervous system. Since hypothalamic monoamines are closely associated with the occurrence of the PIR (8) AMPH, by acting through these central sites, might modulate the vagal output from the brain stem. The absence of an effect of AMPH on insulin secretion in aged rats may be due to a shift in the balance between inhibitory

sympathetic and stimulatory parasympathetic neural input to the pancreas during the process of aging. The inhibitory action of adrenergic neurotransmitters on insulin secretion may be increased in the aged rat. The preabsorptive insulin response was also potentiated by the neuropeptide AVP in young, but not in aged rats. Although AVP may affect directly hepatic glucose release (27), the absence of differences in the blood glucose levels before food presentation suggests that other mechanisms are involved. There is disagreement about the direct effect of AVP on B-cells. Malaisse et al. (14) found no effect of AVP on insulin secretion by B-cells in vitro. More recently Gao et al. (7) showed that AVP produced a dose-dependent amplification of glucose-induced insulin release in normal mouse islets. Since AVP had little effect on cAMP levels, but increased inositol phosphate levels in islet cells, it was concluded that this AVP evoked amplification involves a stimulation of phosphoinositide metabolism. The differential effect of AVP on insulin secretion in young and aged rats may therefore be based upon a difference in the sensitivity of the B-cell second messenger system. AVP also acts on central nervous systems (28), therefore, an age-related change in central vasopressinergic systems might also be responsible for the differential effect of AVP on the PIR.

While the drug and the peptide did not enhance the insulin response in aged animals, both treatments did restore the bradycardiac emotional stress response in aged rats in doses as used in this experiment (18). Several factors may contribute to this difference. An increased behavioral arousal by AMPH and AVP (10,15,28) probably plays a permissive role in the stress evoked bradycardia (18). So far, there is no evidence that behavioral arousal is a causal factor in eliciting the PIR. An alternative explanation may be based upon a differential innervation pattern of the pancreatic B-cells and the heart by the vagus nerve. Innervation of the former originates mainly in the dorsal motor nucleus of the vagus (13). Vagus cardiac preganglionic cells have been localized in the nucleus ambiguus, the dorsal motor nucleus of the vagus and in an intermediate zone between these two nuclei (16,17). One can also not exclude the possibility that the responsiveness of the B-cell cholinergic (muscarinic) receptors to vagal stimulation is exclusively diminished.

In summary, the present study suggests a decrease in parasympathetic influence on the early insulin response to a meal in aged rats. In contrast to a condition with an aversive stimulus, AMPH and AVP were not able to reinstate the vagal responses in aged rats during the nonaversive stimulus presented here.

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Chapter 5

NEUROENDOCRINE AND CARDIOVASCULAR RESPONSES TO MILD STRESS IN YOUNG AND AGED RATS

B. Buwalda, S.M. Korte, G.A.H. Bouws, J.M. Koolhaas and B. Bohus

ABSTRACT

Under resting conditions, mean arterial pressure (MAP) and heart rate (HR) were not significantly different in free moving, naive 24 mo old male Wistar rats when compared to young adults (3 mo old). Basal levels of plasma norepinephrine (NE) and corticosterone (CORT) were elevated in the aged animals, while epinephrine (E) levels were the same order of magnitude in both age groups. Mild stress, consisting of transportation to an open field where a sudden silence was imposed on background noise, caused similar cardiovascular responses, -i.e. increase in MAP and HR - in young and aged rats. Plasma NE and CORT responses to stress were slightly, but not significantly blunted in aged rats. Plasma E responses were in the same order of magnitude in both ages. Post-stress recovery rates of MAP and HR were similar in young and aged animals, while recovery of plasma NE response was delayed in aged rats.

The present findings indicate that tonic sympathetic and adrenocortical activity is elevated in aged rats, while post-stress recovery rate of plasma NE appears to be delayed in aged rats. The cardiovascular and endocrine responsiveness to mild stress, however, is little affected by age.

INTRODUCTION

It is well recognized that stimuli which provoke changes in emotionality have a strong impact on the neuroendocrine and autonomic nervous system (3,13,25). The character of the stress response is the result of interactions between the environment (controllability/ predictability), the coping strategy , the properties of the stressor and bodily factors (4). Aging represents an important variable in stress responsiveness. Previous studies in this laboratory have focussed on the age-related changes in the autonomic stress-responses due to stressors inducing behavioral immobility. During such immobility - i.e. passive way of coping, young male Wistar rats react predominantly with a parasympathetic, cardio-inhibitory response (2,3,19,28). This stress-induced bradycardia, which is a relative change in heart rate in comparison to non-punished free moving male rats, appears to be absent in aged rats (28). It was suggested that an age-related reduction of vagal responses might be associated with a deficit in the immediate evolvement of neuronal activity underlying behavioral and autonomic arousal (28). Arousing or stressful events are well known to activate both the pituitary adrenocortical axis and the sympatho-adrenomedullary system resulting in raised plasma concentrations of the adrenal corticosterone and of the catecholamines norepinephrine and epinephrine (1,13). Since sympathetic output is modulated prejunctionally by vagal acetylcholine (27), the diminished vagal stress responsiveness may lead to a disturbed autonomic balance in aged rats. Although the relation between aging and physiological stress responses is receiving increasing attention, only a few studies have examined autonomic and endocrine responses to emotional stress in freely moving aged rodents (9,26). Accordingly, it is of interest to investigate these stress responses, in particular to less demanding situations, in order to find out whether the age-related reduction of vagal

stress responses (28) is also reflected in sympathetic as well as endocrine responses.

In this paper young and aged rats' cardiovascular, neuroendocrine and autonomic stress responses to a short mild emotional stressor are presented and evaluated. Stress consisted of transportation to a different environment where a sudden silence was imposed on background noise. Former observations showed that this type of stressor causes pronounced cardiac and behavioral responses in young male Wistar rats (8,19).

METHODS

Animals

Young (3 mo old) and aged (24 mo old) male Wistar rats (weighing resp. 298 ± 3 and 514 ± 53; originating from CpB TNO, Zeist, The Netherlands and bred in this laboratory) were housed individually in clear Plexiglas cages (25x25x30 cm) on a 12h light-dark regime (light on between 07.30h - 19.30h) at a room temperature of 21 ±2°C. All animals had free access to standard food (Hope Farms rat chow) and water.

Surgery

All surgery was performed under complete ether anesthesia at least one week prior to testing.

Venous catheter. The animals were provided with a permanent silicon catheter (0.95 mm OD., 0.50 mm ID.) in the right atrium inserted via the right jugular vein and externalized on the top of the skull according to the techniques described earlier (35). The rats were provided with these catheters to allow frequent blood sampling in unrestrained and undisturbed freely moving rats (37).

Arterial catheter. For direct recording of arterial blood pressure and heart rate the rats were also provided with a catheter in the descending aorta according to slight modifications of techniques described earlier (7,36). The aorta was exposed via a midline incision in the abdomen. Aortic blood flow was briefly stopped by application of a small artery clip rostral to the level of the iliolumbar vessels. A silicon catheter (0.95 mm OD., 0.50 mm ID.) with a "J" shaped teflon tip (TW30, Talas, Ommen, The Netherlands), oriented in an upstream direction, was inserted through a 23 gauge needle puncture into the abdominal aorta. The puncture was made approximately 0.3 cm rostral to the bifurcation of the aorta. The length of the teflon tubing in the aorta was ±2 cm. After insertion the catheter was anchored to the left psoas muscle, just lateral to the aorta. No leakage occurred at the point of insertion, the elasticity of the aortic wall being sufficient to close the wound around the catheter. Like the venous catheter the arterial catheter was externalized to the top of the skull and filled with a 50 percent heparinized polyvinylpyrrolidonum (M = ±25000) (PVP) solution (35). This PVP solution was refreshed daily.

Experimental procedure

All experiments were performed between 09.00 and 13.30 hr, -i.e. in the period of stable and low plasma levels of E, NE and CORT (12). To expose the animals to a mild stress of environmental change the rats were transferred to an open field of clear Plexiglas measuring 85x60x60 cm, for a period of 5 min on Day 1. The floor was covered with wood shavings. A constant background "white" noise (65 dB, 2-8 kHz) produced by a noise generator was also provided in the field. The open field was located in an experimental room acoustically isolated from the animal housing. On Day 2, i.e. the test day, the animals were connected to polyethylene tubes (±0.4 m length, 1.45 mm OD. and 0.75 mm ID.) for blood sampling and cardiovascular monitoring at least 45 min prior to testing. At t=0 min the rats were exposed again to the open field. During the first 2 min the background noise was on but was then switched off, leaving the chamber in silence for the final 3 min. After the fifth min the rats were transferred back to their home cages.

Blood sampling and cardiovascular monitoring

Blood samples of 0.5 ml were taken in the home cage (at 15 min before transportation to the open field (baseline) and at t=20 min) and in the open field (at t=1.5, 2.5 and 4.5 min). After each sample the same quantity of heparinized donor blood (25 units per ml) was given in order to minimize the changes in blood volume with related changes in hemodynamics (35). Donor blood was obtained from unstressed rats with permanent heart catheters.

Before the onset of the experiments mean arterial pressure (MAP, mm Hg) levels were calibrated by applying water pressures to the transducer. The connecting tube was filled with heparinized saline (10% heparin of 500 IU/ml). MAP and heart rate (HR, beats/min) were measured in the home cage (30 sec recordings) immediately prior to blood sampling at t=-15 and t=20 min. In the open field measurements were taken at t=1, 2 and 4 min. All recordings in the open field were continuous. The data were calculated from 30 sec samples except of the t=2 min. This 10 sec period represented the immediate stimulus change, -i.e. switching off the noise.

Cardiovascular data acquisition

Arterial blood pressure was recorded via a pressure transducer (Honeywell 130 PC) with an amplifier (Electronics Service, Biological Center, Haren, The Netherlands) and an analog-to-digital converter (RTI-800, Analog Devices, Inc.). The pressure transducer was placed at the level of the heart. Interbeat intervals were measured from the pulse wave. The signal was fed into a microcomputer (Olivetti M24) for data processing and display. The blood pressure was analog-to-digital converted (12 bits) at a rate of over 1.0 kHz. Data processing and display was performed by the CARDIA software package (F.W.Maes, in preparation). HR in beats per min was calculated from interbeat intervals. Following each heart beat, high and low peak values for arterial blood pressure (systolic and diastolic pressures, in mm Hg) were determined. MAP was calculated as (systolic pressure + 2x diastolic pressure)/3. The data acquisition loop of the main program had a cycle time of not more than 1.0 millisecond. This included an on-line graphical representation of data on screen and resulted in a numerical dump of data on floppy disk for subsequent numerical and graphical analysis.

Chemical determinations

Blood samples of 0.45 ml were withdrawn for determination of plasma epinephrine (E), norepinephrine (NE) and corticosterone (CORT). The samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.01 % EDTA as antioxidant and 10 μl heparin solution (500 IU/ml) as anticoagulant. Blood was centrifuged at 4°C for 10 min at 5000 rpm, and 100 μl of the supernatant were stored at -20°C for corticosterone and at -80°C for the catecholamine measurements. Plasma corticosterone (CORT, μg/dl) was measured by means of reversed phase high performance liquid chromatography, as described earlier (11). Determination of plasma catecholamine concentrations was performed by HPLC in combination with electrochemical detection (ECD) as described earlier (33), with minor modifications.

Statistics

Results are presented as means ± SEM. For statistical analysis of data Multivariate Analysis of Variance (MANOVA-Stats program) with repeated measures was used (5 levels). MANOVA was followed by two-tailed t-tests. The paired t-test was used for comparisons within individuals. A probability level of $p<0.05$ was taken as statistical significance.

RESULTS

Arterial pressure and heart rate

Fig.1 shows MAP before, during and after mild stress of stimulus change. Basal MAP in the aged rats was 123.5±4.5 mm Hg. Although this value was higher than the MAP in young rats (114.8±3.4 mm Hg), the difference was not significant. Placement in the open field significantly increased MAP in the young rats (9.9 ± 3.5 mm Hg). The increase of MAP in the aged animals (7.9 ± 3.5 mm Hg) was the same order of magnitude. Switching off the background noise did not cause a further elevation in MAP neither in young nor in aged rats. Home cage measurements 20 min after the stress showed that post-stress recovery was similar in young and aged animals. Both groups regained pre-stress basal MAP levels.

Fig.1 also depicts measures of HR before, during and after the stress of stimulus change. Basal HR was slightly but not significantly higher in young rats (345±34 beats/min) compared to aged rats (329±43 beats/min). HR increased to transfer to the open-field were the same order of magnitude in young (75 ± 12 beats/min) and aged (68 ± 19 beats/min) rats. A different pattern of changes was seen after auditory stimulus change: a slight, but significant ($p<0.05$) decline was seen in the aged rats but not in the young ones. Home cage measurements of HR 20 min after the stress in young and aged rats were not different from pre-stress basal levels.

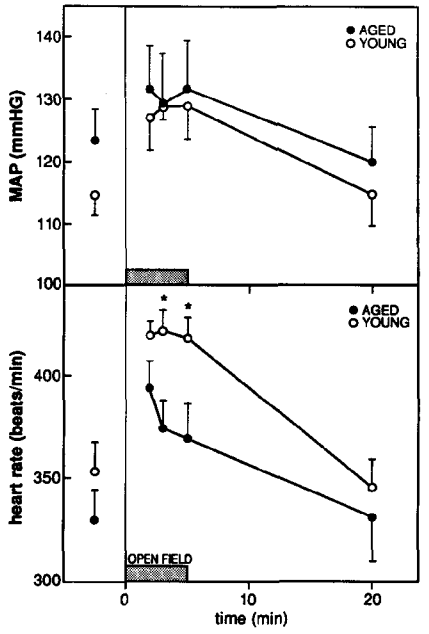

Fig.1.
*Mild stress and changes in mean arterial pressure (MAP, upper panel) and heart rate (lower panel) of young -o- (3 mo old) and aged -●- (24 mo old) rats. A 5 min exposure to an open field is indicated by the shaded area on the horizontal axis. The background noise was switched off immediately before the second sampling in the open field. The values before 0 min, and at 20 min were taken in the home cage. Means ± SEM are plotted from 8 animals in both groups. * $p<0.05$ (two-tailed t-test).*

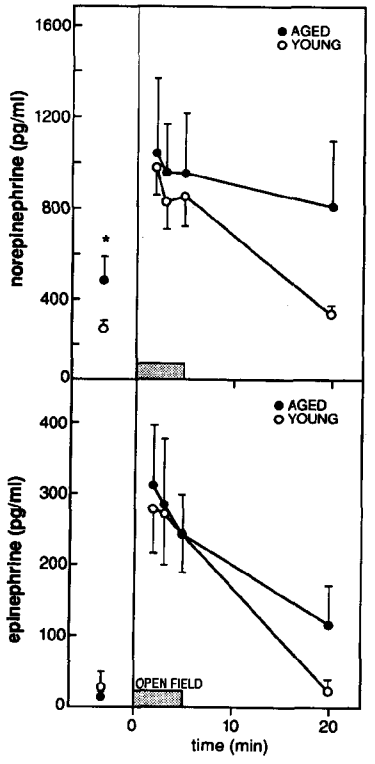

Fig.2.
Mild stress and changes in plasma norepinephrine (NE) and epinephrine (E) in young -o- (3 mo old) and aged -●- (24 mo old) rats. Means ± SEM from 9 animals in the young and from 7 in the aged group are shown.
** $p<0.05$ (two-tailed t-test).*
For further explanation see Fig.1.

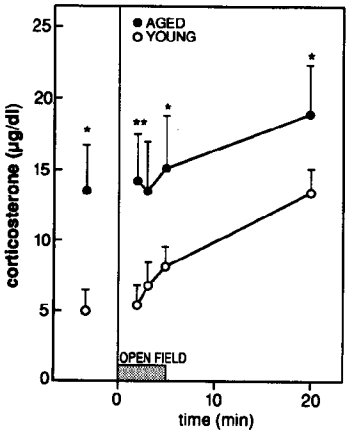

Fig.3.
*Mild stress and changes in plasma corticosterone (CORT) in young -o- (3 mo old) and aged -●-(24 mo old) rats. Means ± SEM from 9 animals in the young and 7 in the aged group are shown. * $p<0.05$, ** $p<0.01$ (two-tailed t-test).*
For further explanation see Fig.1.

Norepinephrine

Fig.2 shows plasma levels of NE before, during and after the stress experience in the open field. Basal levels of NE were significantly higher ($p<0.05$) in aged rats (491±99 pg/ml) compared to young ones (253±35 pg/ml). MANOVA for the repeated measures showed a significant increase in plasma NE levels to the stress situation in young ($F(4,5)=9.5$; $p=0.0003$) as well as in aged rats ($F(4,4)=3.1$; $p=0.04$). There was no significant effect of age, nor was an interaction seen between age and sampling time. Switching off the noise did not cause a further change in NE levels. The rate of recovery of the NE response to stress was measured by comparing peak values at t=1.5 min with home cage values at t=20 min. In young rats NE levels rapidly fell 658 ± 111 pg/ml which was significantly higher ($p<0.01$) than the fall in aged animals (236 ± 41 pg/ml). This difference indicates a delayed recovery in the aged group.

Epinephrine

Fig.2 also shows plasma levels of E before, during and after stress. Basal levels of E were the same in young (29.8±19.4 pg/ml) and aged rats (13.3±8.6 pg/ml). MANOVA for the repeated measurements showed a significant increase in plasma E levels both in young ($F(4,6)=7.2$; $p=0.0008$) and aged rats ($F(4,3)=5.6$; $p=0.009$). There was no significant effect of age, nor was an interaction seen between age and sampling time. Switching off the background noise did not cause further change in E levels. At t=20 min E levels in aged rats were slightly higher than in young rats. This difference was close to significance ($p<0.08$). Recovery rates, however, were not significantly different between young and aged rats.

Corticosterone

Fig.3 shows plasma CORT levels before, during and after stress. Basal level of CORT in aged rats (13.5±3.2 μg/dl) was significantly higher ($p<0.05$) than the one seen in young adults (5.1±1.4 μg/dl). While both the young ($F(4,8)=19.6$; $p<0.000$) and aged ($F(4,4)=4.1$; $p=0.02$) group showed a significant increase in time of plasma CORT levels, MANOVA indicated a significant group difference ($F(1,12)=7.4$, $p=0.02$) and a significant interaction between age and sampling time ($F(4,48)=3.8$; $p=0.01$). This interaction probably is caused by a delayed and diminished CORT response in aged rats. However, the maximal plasma CORT responses which were reached in the home cage at t=20 min in both age groups were not significantly different between young (8.7 ± 1.9 μg/dl) and aged (5.6 ± 2 μg/dl) rats.

DISCUSSION

In the present study differences between young and aged rats' baseline sympathetic and adrenocortical activity were observed, while cardiovascular and endocrine responsiveness to a mild stress situation were not significantly different in aged animals. Post-stress recovery rate of plasma NE was delayed in aged rats.

Resting MAP is slightly elevated while HR shows a tendency to be lower in aged rats, but the differences failed to reach significance. Conflicting data are available for MAP and HR in aged rats. Increased basal MAP was reported by Chiueh et al. (9), others (16,26) failed to observe changes in this parameter. One of the possible variables in causing these different findings may be the increasing heterogeneity in a group of aged animals. This is reflected in the larger SEM. Another variable may be the previous "stress history" of the animals. In a study concerning conditioned stress responses subsequent to this experiment (22), the same aged rats showed a significantly higher basal MAP than the young ones. The difference could be ascribed to a decreased basal MAP in the young animals. This may mean that old male rats less easily adapt to handling and other experimental procedures as far as the blood pressure regulation is concerned. The finding that resting HR falls with age is consistent with one study (9) but conflicting with others that report no change (16) or an increased HR in aged rats (26).

Pre-stress basal plasma levels of NE but not of E were elevated in aged rats. This finding confirms earlier reports (9,32). Higher levels of plasma NE may reflect an age-related decrease in the metabolic clearance rate, due to changes in regional circulation (17). Most data, however, suggest an age-related increase of NE spillover (32). Such an increase might be of physiological significance because of decreased responsiveness of end organs with age (20).

Pre-stress basal plasma levels of CORT were also elevated in the aged rats which is in agreement with a number of former reports (15,24,31). Two types of receptors for corticosterone in the brain appear to be involved in the regulation of ACTH and corticosterone secretion (29). Since hippocampal type I receptors are occupied even with low circulating titers of CORT (30), it is tempting to speculate that the type I receptor mediates a tonic inhibitory influence on the HPA axis (10,14). Some studies report a decrease in hippocampal type I receptors in aged rats (21,24). The diminished inhibition of CORT release might be a cause for the observed increase in basal CORT levels in the aged rats.

The response of the cardiovascular system to transport and placement in an open field is not markedly different in aged rats.

The age-related difference in NE and CORT stress response failed to reach significance. This probably is caused by the large variability in responsiveness in aged rats. In the study of Korte et al. (22) subsequent to this experiment this variability was reduced and NE responsiveness to conditioned stressors appeared to be diminished in aged rats. In the present experiment the slightly blunted NE and CORT responses to transportation assume an age-dependent change in stress-induced arousal. Due to the large variability in aged rats this assumption, however, needs further extended investigation involving larger groups of animals.

Post-stress recovery of plasma NE response was delayed in the aged rats which is in agreement with other studies in rats (26). A delayed recovery can be caused by a decreased clearance rate of NE from the blood. Borton and Docherty (5) reported a decreased systemic neuronal uptake of noradrenaline in aged rats. Since an increase in NE uptake was seen in the hindlimb of aged rats (20), there may be tissue-specific alterations in reuptake mechanisms during aging. Studies in humans report age-related decreases in clearance rate, due to changes in regional blood flow (17,18). A prolonged release of NE can also be a cause of a delayed recovery. This may be caused by a decreased negative feedback through prejunctional alpha-2 adrenergic receptors (6). A final possibility is that the sluggish central catecholaminergic response to stress in aged rats is reflected not only in a diminished CORT and NE response but also in a slower inhibition of the plasma hormone responses. Whether the differences seen between young and aged rats' endocrine parameters are developmental in nature can only be solved by studying rats of intermediate age.

The present experiments were designed to study the dynamics of the physiological and endocrine stress response to the sudden, but mild stress of background auditory stimulus change. Former behavioral and cardiac rate studies have justified the use of this paradigm both in young and aged rats (8,19,34). Surprisingly, not only a slowly reacting system like the pituitary-adrenal axis failed to change rapidly, but also blood pressure, heart rate and plasma catecholamine responses were absent. Responses to transfer and/or to the novel environment were very marked, however, at all parameters studied here. The absence of a dynamic response to the sudden stimulus change probably can be explained by the different experimental conditions in this study. It occurred to us that the orienting response to the mild stress of a sudden change in the environment is very sensitive to distracting stimuli like

sound and movement. Heart rate responses formerly studied to the occurrence of sudden silence were always biotelemetrically monitored with minimal disturbance of the animal. In the present study the rats were connected to tubes for blood sampling and cardiovascular monitoring and two experimentators had to stand next to the open field. This situation most likely provided too much distracting factors, inhibiting behavioral and physiological responses to switching off the background noise

Together with the previous studies concerning the age-related reduction of vagal stress responses, the present findings indicate that the decrease in parasympathetic stress responsiveness in aged rats not necessarily leads to a disturbed balance in autonomic stress responses indicating that in aged rats probably compensatory mechanisms evolve. Whether these processes occur in the central nervous system or by adaptations in peripheral organs needs further investigation.

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Chapter 6

EFFECTS OF NEONATAL ADMINISTRATION OF VASOPRESSIN ON CARDIAC AND BEHAVIORAL RESPONSES TO EMOTIONAL STRESS IN ADULT MALE RATS

B. Buwalda, C. Nyakas, J.M. Koolhaas and B. Bohus

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ABSTRACT

Arginine-8-vasopressin (AVP) was administered subcutaneously on postnatal days 3-7 in a high (10 μg/100 g b.w.) or a low dose (1 μg/100 g b.w.) to male Wistar rats. Control pups were untreated or saline injected. Behavioral observations in a complex maze after maturation indicated that neonatal administration of AVP increases exploratory behavior in this novel environment in a dose-dependent way. Cardiac monitoring during the conditioned emotional stress of fear of inescapable electric footshock showed that only the high dose of AVP attenuates the bradycardiac stress response. The analysis of cardiac responses also suggested an adult hyposensitivity to AVP in rats treated neonatally with AVP. In addition, the low dose of neonatal AVP was impairing the retention of a passive avoidance behavior.

The data indicate that the neonatal administration of AVP exerts long-term effects upon the behavioral adaptation to novelty and memory processes related to emotional stress. That neonatal AVP is less effective in influencing adult vagally mediated cardiac stress responses suggests differences in the developmental sensitivity ("critical periods") of the central vasopressinergic systems involved in the regulation of behavior and autonomic functioning.

INTRODUCTION

Manipulation of the endocrine environment of the newborn rat may have remarkable impact on the "programming" and "organizational" processes in the developing central nervous system (3,11,20). As a result, long-term changes in a number of physiological and behavioral patterns, including responsiveness to the "activational" effects of hormones, can be observed later in life (9,15).

The neurohypophyseal peptide arginine-8-vasopressin (AVP) is an important modulator of physiological and behavioral stress responses via brain and peripheral mechanisms in adult animals. The facilitating effect of AVP on memory consolidation and retrieval processes has been repeatedly described (6,10,17,19). Reports on the way in which AVP can modulate behavioral activity are not equivocal, probably due to behavioral state- and dose-dependent effects of AVP and differences in route of administration. Centrally applicated AVP is arousing at a low level, while it reduces arousal at high level of activation (7). Peripherally administered AVP facilitates behavioral activity to a novel environment in a low dose (26), while higher doses suppress activity (25). This behavioral suppression may be due to the marked peripheral hemodynamic effects caused by higher doses of AVP. Experiments in our laboratory in freely moving rats suggested that AVP also serves as a modulator of the vagally mediated cardio-inhibitory response to certain emotional stressors (5,8,12,22). The profound effect of neonatally administered AVP on learning and memory task performance in later life (21,27) suggests that the peptide may have important programming and/or organizational effects on brain and physiological processes. In this paper we present evidence that postnatal administration of AVP affects behavioral reactivity

to novelty, the cardiac response to an emotional stressor and adult passive avoidance behavior. In addition, attention has been paid to the effect of neonatal exposure to AVP on the modulating properties of vasopressin on the bradycardiac stress response in adulthood.

METHODS

Animals

Thirty-two male Wistar rats (CpB TNO, Zeist, The Netherlands and bred in this laboratory) were used. Males in litters were culled to 8 pups each. Eight pups remained untreated. The other 24 pups received daily subcutaneous (s.c.) injections, beginning on postnatal day 3 and ending on day 7, of one of the following preparations: (1) saline; (2) AVP1, 1 μg/100 g bodyweight; (3) AVP10, 10 μg/100 g. AVP was dissolved in saline. The assignment of pups within a litter to treatment groups was done at random. Each mother raised pups of a variety of treatments. The pups were weaned on day 23 and housed 5 to a cage at a standard temperature of 21 ± 2°C in a light-controlled room (light on between 07.00-19.00h). All animals had free access to standard food and water. The behavioral and cardiac tests were performed at an age of 3 months old between 9.00 and 13.00 hr.

Surgery

In order to record the electrocardiogram (ECG) two transcutaneous stainless steel electrodes made of standard paperclips were implanted under light ether anesthesia by a technique described earlier (4). Two days of recovery were allowed after this minor operation.

Behavioral Reactivity to Novelty

Apparatus

Novelty-induced behavioral reactivity was tested in a complex maze. The apparatus was a modified Hebb-Williams (60x60x60 cm) maze made of plywood and covered by wire mesh. The maze was divided into alleys and subfields by means of wooden falls. The floor of the maze was divided into 10x10 cm squares. The maze was placed in a sound-attenuated semi-dark room.

Procedure.

In the complex maze the behavior of each rat was observed for 5 min. The number of floor units entered and the number and duration (sec) of rearings were recorded with the aid of a microprocessor. This behavioral test always preceded the study in the emotional stress situation.

Emotional Stress, Behavior and Cardiac Response

Apparatus

A step-through type passive avoidance apparatus as designed by Ader et al. (1) was used to investigate emotional stress-related behavior and cardiac response. Briefly, the apparatus consisted of a dark compartment (40x40x40 cm) and a well lit platform attached to the front center. A small sliding door separated the two compartments. An unavoidable painful electric footshock could be delivered in the dark box through the stainless steel bars which served as a floor.

Recording and analysis of the ECG

The ECG of freely behaving rats was monitored telemetrically by means of a miniature FM transmitter (model SNR 102F, Dynamic Electronics Ltd., London, England), attached to a velcro strap secured around the chest of the rat (4). The transmitted signals were received on a commercial FM receiver, amplified (Grass P5CR preamplifier) and stored on tape with the aid of an instrumentation recorder (Minilog, Philips) for off-line computer analysis. Prerecorded ECG samples were played back through a cardiotachometer pulsegenerator which generated a square wave electric pulse at each R wave. The time between the onset of two consecutive pulses, the interbeat interval (IBI), was measured by a personal computer (Olivetti M24). IBI's shorter than 100 and longer than 220 msec were discarded because these were likely to be due to artefacts. The heart rate was expressed as mean IBI: the longer the IBI, the lower the heart rate was.

Experimental procedure

The animals were first habituated to the experimental circumstances. Immediately before each of the daily sessions the strap holding the transmitter was fixed around the chest of the rat in the animals' room. After transportation to the experimental room, on day 1 the rat was allowed a 3-min adaptation to the dark compartment. Immediately afterwards a single training trial was given during which the animal was placed on the illuminated platform and allowed to enter the dark compartment. The sliding door was closed and the rat stayed another 3 min in this compartment. On days 2 and 3 the training procedure was repeated. The rat was directly placed on the platform. Following entering the dark compartment the rat was removed after 5 min. To accustom the animals to the injection method, s.c. saline injections were given 1 hour prior to the training on these days. At the 4th daily session ECG recordings started in order to obtain prestress heart rate values. Sixty min prior to the test, the animals were saline injected (s.c.) to achieve correct control values for poststress recordings. The ECG recording lasted for a period of 1 min in the dark compartment, starting immediately upon entrance. In a second session on day 4, an inescapable electric footshock (0.6 mA, 2 sec) was delivered immediately after entering the dark. The rat was removed after 1 min and returned to the home cage. To obtain postshock ECG values under emotional stress of fear of inescapable footshock, the rats were subjected to the experimental situation on days 5 and 6. They were placed directly into the dark compartment with closed sliding door. ECG's were recorded in the first min of the exposure. On these two days animals were injected subcutaneously with saline or AVP, in a dosage of 3 μg/kg, with a cross-over design. Accordingly, each animal served as its own control. The treatments were given 60 min prior to testing.

Avoidance latency

The latency of entering the dark compartment from the lit platform was used as the behavioral measure of the conditioned emotional behavior. This, usually designated as one-trial learning inhibitory or passive avoidance test was performed 1 week after the two short forced exposures to the dark compartment. The rat was placed on the platform facing away from the open sliding door to the dark compartment. The latency to reenter the dark was measured up to a maximum of 300 sec. No treatment was given at this time.

Statistical analysis

Postnatal saline injections did not significantly influence cardiac and behavioral responses as compared to untreated animals. Results obtained in untreated and neonatal saline treated rats were therefore pooled in a so-called control group. Avoidance latencies were expressed individually and as median latency in sec. The other results were calculated as means ± SEM. Cardiac data were analyzed using a one-way ANOVA and a paired t-test. Behavioral data were evaluated for significance using the non-parametric Kruskal-Wallis ANOVA and the Mann-Whitney U-test. A probability level of $p<0.05$ was taken as statistical significance for all tests.

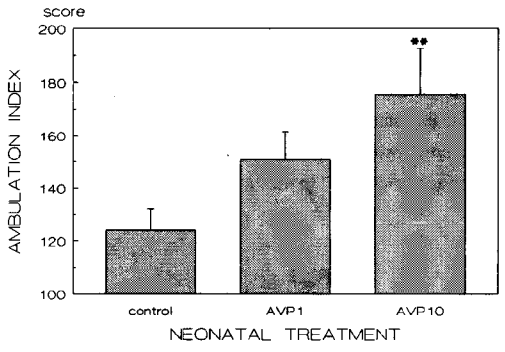

Fig.1

*Behavioral reactivity to novelty in a complex maze of control (8 neonatally untreated and 8 saline treated) rats and of rats treated neonatally repeatedly with a low (AVP1; n=8; 1µg/100g b.w.) or a high (AVP10; n=8; 10 µg/100g b.w.) dose of AVP. Ambulation index is a combined measure of rearing and crossing activities ((1/2 x number of crossings) + (number of rearings + duration of rearings)). **p<0.01 vs. control (Mann-Whitney U-Test).*

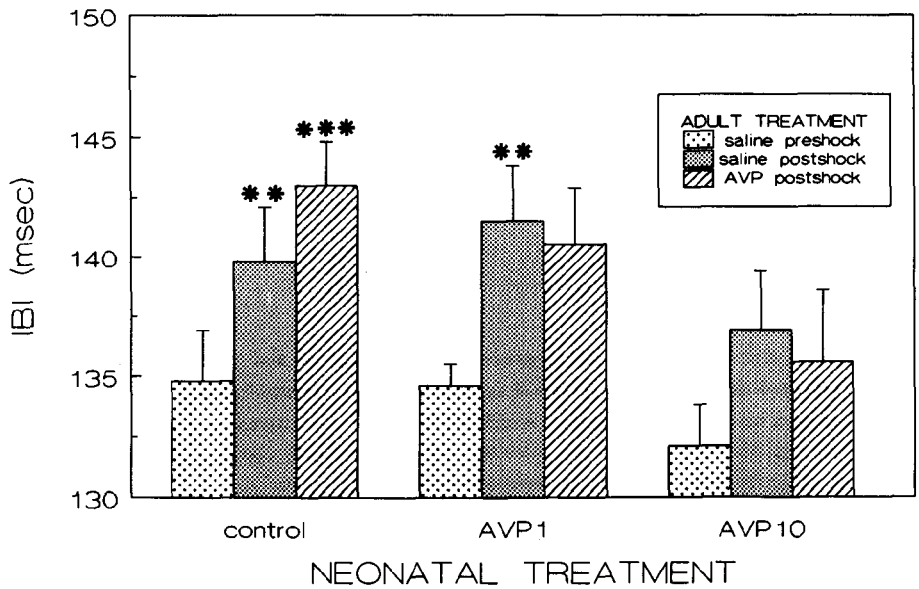

Fig.2

Heart rate expressed as interbeat interval (IBI) before (preshock) and one day after inescapable shock (postshock) in adult control animals; animals treated neonatally repeatedly with a low (AVP1) or a high (AVP10) dose of AVP during the first min of an emotional stress of forced exposure to the dark compartment of a passive avoidance apparatus where the footshock was delivered earlier. Preshock measurements were performed after saline injection one day before footshock was delivered. Prior to the two postshock tests the animals were injected in a cross-over design with saline and AVP (3 μg/kg). The substances were administered 60 min prior to testing. *p<0.05; **p<0.01; ***p<0.001 vs. preshock (paired t-test). For further details see Fig.1.

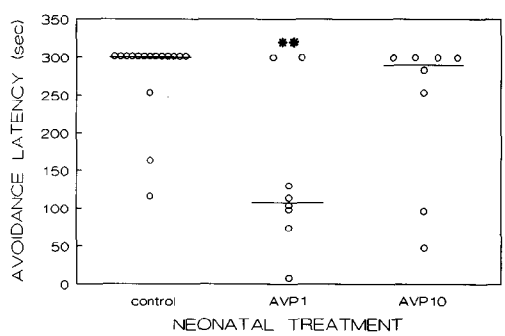

Fig.3

Avoidance latencies one week after inescapable footshock of controls and rats treated neonatally with AVP. Results are presented as individual scores and as medians in sec. **p<0.01 vs. control (Mann-Whitney U-test). For further details see Fig.1 and 2.

RESULTS

Behavioral Reactivity to a Novel Environment

Fig.1 shows that neonatal AVP administration dose-dependently increased adult activity in the complex maze compared to the saline treated group ($H(2,32)=7.99$, $p=0.01$).

Cardiac and behavioral responses to emotional stress

Fig.2 shows the mean cardiac rate in preshock conditions after adult saline administration and in postshock conditions after the rats were treated with saline or AVP. Neonatal AVP administration had no effect on heart rate under preshock conditions.

As compared to the preshock condition the emotional stress of the exposure to the former shock compartment was accompanied by longer mean IBIs in the control group, suggesting a relative bradycardiac response ($p<0.05$). AVP administration prior to the test caused a further increment of the bradycardiac stress response ($p<0.001$). Rats receiving neonatal low doses of AVP (AVP1) showed a bradycardiac stress response similar to the one performed by the control animals. However, adult pre-test AVP administration failed to cause a further increase in the magnitude of the stress bradycardia in this group. The group receiving neonatal high doses of AVP (AVP10) did not show a significant bradycardiac response to stress, neither under saline nor AVP treatment conditions. One-way ANOVA failed to reinforce a significant neonatal treatment effect on cardiac stress response (postshock IBI- preshock IBI) following saline ($F(2,29)=0.23$, $p=0.8$) or AVP administration ($F(2,29)=1.12$, $p=0.3$). ANOVA also indicated that there was no neonatal treatment effect on absolute IBI in both preshock and postshock conditions.

Fig.3 depicts the avoidance latencies measured one week after the last cardiac recording session. Rats receiving neonatal repeated injections of the lower dose of AVP showed significantly shorter avoidance latencies than the controls ($p<0.01$). Avoidance latency in rats treated with the high doses of the peptide was not impaired.

DISCUSSION

The present findings suggest that increased availability of vasopressin during the first week after birth markedly affects adult memory to aversive events and behavioral reactivity in a novel environment. The adult cardiac response to an emotional stressor appears to be less sensitive to neonatal AVP treatment.

The analysis of cardiac stress responses suggests that an adult hyposensitivity to AVP may exist in rats treated neonatally with AVP. This suggestion, however, needs further extended investigation. While neonatal treatment with the lower doses of AVP did not alter

the magnitude of the stress bradycardia, this dosage clearly affected memory retrieval as indicated by the impaired avoidance behavior performed by these animals. The results presented here suggest that vasopressinergic systems involved in autonomic and behavioral stress responses are differentially affected by neonatal administration of AVP. In our view, at least two possible explanations may be given. The first may be the late development of tonic parasympathetic control of the rat heart. The vagal regulation of cardiac rate appears to be complete only after the second week of postnatal life (16). To manipulate the vagally mediated bradycardia to stress by neonatally administered AVP, it is perhaps necessary to extend or change the period of administration. The second explanation may be a different critical period to endocrine manipulation for the diverse central and peripheral vasopressinergic systems. This might originate from a differential development in time of vasopressinergic systems involved in autonomic and behavioral processes. As to central vasopressinergic systems it is known that the first appearance of AVP binding sites during embryonic life is coincident with the first detection of AVP mRNA and of immunoreactive AVP (2,18). Petracca et al. (23) showed, however, that in the amygdala, binding did not change after postnatal day 3, while binding sites in the septum proliferated slowly to attain adult (90 days) distribution. Tribollet et al. (28) also showed that the appearance of AVP receptors is not the similar in many areas of the rat brain and depends on the developmental stage.

The impaired passive avoidance behavior of animals treated neonatally with the low doses of AVP may be interpretated as a decreased sensitivity in adulthood of vasopressinergic systems involved in memory processes to endogenous vasopressin. It is generally accepted that AVP affects passive avoidance behavior (13,19,24). Differences in findings about facilitation or inhibition of avoidance retention by vasopressin are often dose or arousal related (7,13). This might be reflected in the failure of the high doses of AVP to impair later avoidance retention. Handelmann and Sayson (14) showed that postnatal injections of vasopressin decrease the number of binding sites for AVP in the adult kidney. This receptor downregulation probably also occurs in the certain brain areas. Chronic prenatal administration of AVP also impairs adult memory retrieval (27), suggesting a decreased receptor sensitivity to endogenous vasopressin. If Bohus' (7) hypothesis that certain vasopressinergic systems serve behavioral passivity is correct, the observed behavioral changes in the novel environment may also be attributed to a hyposensitivity of central vasopressinergic systems. However, the exact mechanism underlying the differential physiological and behavioral effects of neonatal administration of AVP needs further studies.

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Chapter 7

VASOPRESSIN DELAYS RECOVERY OF BEHAVIORAL AND CARDIAC RESPONSES TO MILD STRESS IN YOUNG BUT NOT IN AGED RATS

B. Buwalda, C. Nyakas, J.M. Koolhaas, P.G.M. Luiten and B. Bohus

ABSTRACT

In young male Wistar rats sudden silence superimposed on low intensity background noise evokes a relative decrease in heart rate. This bradycardia is accompanied by immobility behavior. In the present study, involving young (3 mo), late-adult (14 mo), aged (20 mo) and senescent (25 mo) rats the magnitude of the stress-induced bradycardia shows an age-related reduction while the behavioral immobility response remained unchanged during the process of aging. Arginine-8-vasopressin (AVP, 6 μg/kg s.c) administered 60 min prior to the experiment led to a prolonged behavioral and cardiac stress response in young and late-adult rats, but not in aged and senescent animals. The peripheral and central mechanisms possibly involved in the failure of systemically applied AVP to improve bradycardiac stress responses in aged rats are discussed.

INTRODUCTION

The neurohypophyseal peptide arginine-8-vasopressin (AVP) modifies various forms of learned behavior, apart from its well-known classical endocrinological actions. Several studies showed that both systemically and centrally administered AVP prolongs extinction and improves consolidation and retrieval processes in avoidance learning tasks (10,19,23,25,37). A number of studies have focussed on the effects of AVP on physiological responses related to emotion and behavioral adaptation (4,5,20). Experiments in freely behaving adult rats in various stressful situations showed that AVP serves as an important modulator of a bradycardiac response to an emotional stressor (4,20). Since this stress-induced bradycardia appears to be vagally mediated (8), these experiments suggest that AVP is involved in a neural network that serves a parasympathetically regulated response to stress.

Previous research in this laboratory focussed on the age-related changes in the autonomic responses due to stressors inducing behavioral immobility (30). During this passive way of coping, young male Wistar rats reacted predominantly with a vagally mediated cardio-inhibitory response. While the behavioral responsiveness to stress remained intact, the initial bradycardiac response appeared to be diminished in aged rats (30). Therefore, an age-related attenuation of parasympathetic control of cardiac functioning during emotional stress situations was suggested (30). Peripherally applied AVP appeared to reinstate the bradycardiac response to a conditioned stress of fear of inescapable footshock in 14 months old male Wistar rats (5,29). These results led to the hypothesis that AVP dependent mechanisms are involved in the age-related reduction of stress-induced parasympathetic responsiveness. Since the bradycardiac response to conditioned aversive stimuli is thought to reflect the attentional demands of the experimental situation (31), one would expect a comparable action of AVP in non-aversive conditions that result in vagal activation due to acute orientation/attention behavior. Sudden silence superimposed on low intensity background noise is eliciting bradycardia and immediate behavioral arrest in young rats (18). In a previous study AVP was demonstrated to enhance behavioral and cardiac responses to

this "sudden silence" stress in young male rats, whereas it failed to improve or even worsened such responses in 24 months old rats (7).

The aim of this paper was to analyze the modulating properties of AVP on behavioral and cardiac responses to the mild stress of sudden silence not only in young and old rats, but also in rats of intermediate ages to study at which moment a hyposensitivity to vasopressinergic modulation of stress-related behavior and cardiac control develops during the process of aging.

METHODS

Animals and housing

Male Wistar rats of 4 different ages were used. The animals were 4, 14, 20 and 25 months old and originated from the Winkelman substrain (kindly donated by Troponwerke, Cologne, Germany). They were housed 6 to a cage (40x60x15 cm), with food and water ad libitum, in a temperature controlled environment of 21 ± 2 °C; the lights were on from 07.30 to 19.30 hr. All experiments were performed between 9.00 to 13.00 hr.

Surgery

In order to record the electrocardiogram (ECG), transcutaneous stainless steel electrodes made of standard paperclips were implanted under light ether anesthesia. One electrode was placed between the scapulae and the other in the midback region, according to the method described previously (3). At least three days were allowed for recovery before the start of the experiment.

Recording and analysis of the ECG

The ECG of freely moving rats was monitored telemetrically by means of a miniature FM transmitter (model SNR 102F, Dynamic Electronics Ltd., London, England) as described before (3). The transmitter was attached to a Velcro strap secured around the chest of the rat and connected to the transcutaneous electrodes. The transmitted signals were received on a commercial FM receiver, amplified (Narco Bio-System Inc. Mod. FM-1100-7) and stored on tape by a commercial tape recorder. During recording and analysis, the quality of the ECG signal was continuously monitored on an oscilloscope.

Recorded ECG samples were played back through a cardiotachometer pulsegenerator (Schmitt-trigger) that generated a square wave pulse at each R wave. The interbeat interval (IBI) -i.e. the time elapsed between onset of the two consecutive pulses - was measured using a personal computer (Olivetti M24). The mean IBIs were computed for periods of 55 sec. IBIs shorter than 100 and longer than 220 msec were discarded because these were likely to be due to artifacts.

Procedure

The behavioral and cardiac responses to a sudden drop in background noise were measured in a rectangular clear Plexiglas cage (85x60x60 cm), designated as an "open field" in this paper. This open field with a wood shavings covered floor was located in an acoustically isolated experimental room where a noise generator produced a background noise that was maintained at a constant level (65 Db, 2-8 Khz). Upon entering, a Velcro strap holding a miniature FM-transmitter for the ECG recordings was fixed around the chest of the rat. The animal was placed in the open field for 5 min on Day 1. On Day 2, the test day, the animals were exposed again to the open field for 5 min. After 2 min the background noise was switched off, leaving the animal in almost total silence for the remaining 3 min. Heart rate and behavior were recorded for 3 periods (P) of 60 sec. Second min recordings were made during the 60 sec immediately prior to switching off the background noise (P1). Third min recordings (P2) during the 60 sec immediately after switching off the noise (P2) and fifth min recordings (P3) were regarded respectively as response and recovery measurements. As a behavioral measure the time spent "immobile" was determined by an observer during the three periods. Immobility was defined as almost motionless scanning of the environment with only minor head movements.

Treatment

Arginine-vasopressin (AVP) was dissolved in saline and injected subcutaneously (s.c.) in a dosage of 6 μg/kg/ml 60 min prior to the exposure to the open field. Saline injections served as vehicle. The selection of the single dose of the peptide was based on the results of the previous studies of young and aged rats' cardiac response to emotional stress (29) and on several other studies describing behavioral and physiological effects of peripherally applied AVP (20,21,22,24,25). EEG recordings (13) further indicated a similarity between SC administered AVP in a dose of 6 μg/kg and a behaviorally relevant intracerebroventricular administered dose of 1 ng. The rats were injected in a cross-over design with saline or AVP, each animal serving as its own control. A 7-days wash-out period was allowed between injections to minimize interaction of treatments.

Statistics

The results are presented as means ± SEM in two measures; absolute values are presented in the figures and response (P2-P1) and recovery (P2-P3) values in the table. The absolute cardiac data were analyzed using a multivariate ANOVA (two-way) using one between-subjects factor (group) and one repeated measures within-subjects factor (periods), a two-tailed Student's t-test and a paired t-test. Response and recovery values were analyzed using a one-way ANOVA using the factor age. Behavioral data were evaluated for significance using the Kruskal-Wallis ANOVA and the Wilcoxon matched-pairs ranked-signs test. A probability level of $p<0.05$ was taken as statistical significance for all tests.

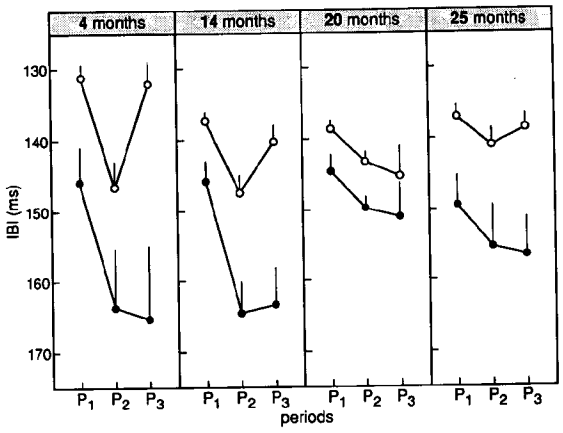

Fig.1. Heart rate expressed as interbeat interval (IBI) during a 5 min exposure to an open field before and after sudden reduction of a non-aversive background "mixed" noise, measured in 4 (n=9), 14 (n=9), 20 (n=8) and 25 (n=11) months old male Wistar rats. One minute recordings were made during the second min of exposure to the open field with the noise on (P1); during the third min immediately after the noise was switched off (P2) and during the fifth min (P3). IBIs are presented after subcutaneous administration of saline (open circles) and AVP (closed circles). Means ± s.e. are shown.

RESULTS

Vasopressinergic modulation of cardiac stress responses

Fig.1 shows the heart rate values, expressed as IBI, of the animals in the open field and the cardiac response to stress of sudden silence. ANOVA testing revealed no effect of age on pre-silence, -i.e. pre-stress values during P1. The magnitude of response as well as the recovery values after sudden cessation of background noise are presented in table 1. Multivariate ANOVA with repeated measures for the three periods showed no significant age effect in vehicle treated animals, but a highly significant interaction between age and periods, ($F(2,64)=4.7$; $p=0.0007$). Although all vehicle treated age groups showed a significant cardioinhibitory response, i.e. an increase in IBI, to switching off the noise, the magnitude of this response (see table 1) showed an age-related decrease, ($F(3,32)=6.27$; $p=0.002$). Recovery, measured as the difference in IBI between P3 and P2 was also reduced in aging, ($F(3,32)=6.73$; $p=0.002$).

Administration of AVP caused a decrease in heart rate already before the reduction of background noise, -i.e. P1, in the open field in all age groups, expressed in a significant treatment effect, ($F(1,65)=21.7$; $p<0.001$). There was no significant interaction between age and treatment on pre-stress values. Multivariate ANOVA for the factors age by treatment by periods also showed no significance. The interaction between age and periods as shown after vehicle treatment disappeared after AVP administration, suggesting an effect of the treatment throughout the periods. This is caused by the absence of recovery during P3 of the cardiac stress response in 4 and 14 months old rats receiving AVP. A significant interaction between treatment and periods was observed in 4, ($F(2,32)=5.88$; $p=0.007$) and 14 months

old rats, ($F(2,32)=3.17$; $p=0.05$). Twenty and 25 months old rats failed to show this interaction.

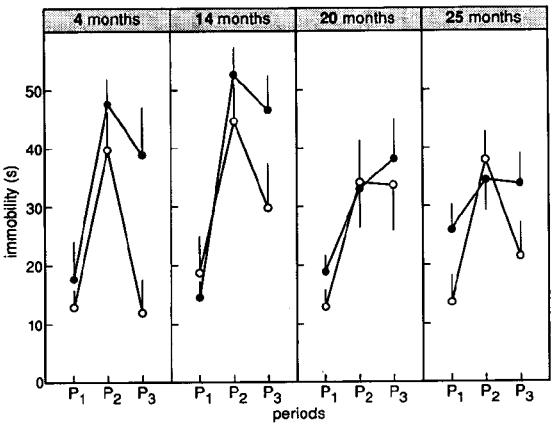

Fig.2. Behavioral immobility in the open field before (P1) and after sudden silence (P2 and P3) in 4, 14, 20 and 25 months old rats.
For further information see Fig.1.

Vasopressinergic modulation of behavioral stress responses

ANOVA testing revealed no significant age or treatment effect on pre-stress immobility values. Neither was an interaction between age and treatment present during P1. Fig.2 shows that all vehicle treated animals responded to sudden cessation of background noise with an increase in immobility behavior (see also table 1). ANOVA (Kruskal-Wallis) indicated that neither the magnitude of the response nor the recovery after the sudden stimulus change were affected by age. Only 20 months old animals failed to show a significant recovery of immobility behavior after stress.

After AVP administration there was an effect of age on immobility responses, ($H(3,37)=14.95$; $p=0.002$). Post-hoc testing indicated that in 14 months old rats AVP increased the immobility response ($p<0.05$), whereas in 25 months old rats the behavioral stress response was reduced ($p<0.01$). A two-way ANOVA showed a significant interaction between the factors age and treatment on immobility responses, $F(3,65)=2.8$; $p=0.04$.

Table 1. *Cardiac and behavioral response values to (P2-P1) and recovery after (P2-P3) the sudden cessation of background noise in 4, 14 20 and 25 months old rats after saline or AVP application.*

	Response to sudden silence		Recovery after sudden silence	
	IBI (msec)	Immobility (sec)	IBI (msec)	Immobility (sec)
4 months				
SAL	15.4 ± 3.7	26.2 ± 3.6	14.1 ± 3.6	28.0 ± 6.3
AVP	18.1 ± 4.2	30.3 ± 5.4	1.7 ± 3.7 ##	8.7 ± 6.0 #
14 months				
SAL	10.0 ± 1.7	34.9 ± 6.1	7.2 ± 1.6	14.7 ± 7.0
AVP	18.8 ± 4.7	37.4 ± 4.4 #	0.9 ± 5.2	6.2 ± 5.8
20 months				
SAL	4.8 ± 1.8	19.0 ± 6.2	-2.1 ± 4.0	-0.1 ± 14.0
AVP	5.2 ± 2.7	13.9 ± 5.4	-0.7 ± 3.8	-5.0 ± 6.2
25 months				
SAL	3.5 ± 0.9	23.7 ± 3.6	2.3 ± 1.1	16.4 ± 4.3
AVP	6.0 ± 3.0	8.7 ± 5.0 ##	-1.2 ± 2.0	0.7 ± 4.4 ##

Within the age groups the response and recovery values after AVP administration were compared with the animals' own vehicle control value by means of a paired t-test (cardiac data) or a Wilcoxon matched pairs-ranked signs test (behavioral data), #p<0.05; ##p<0.01.

DISCUSSION

The present findings indicate that between the age of 14 and 20 months, male Wistar rats develop a hyposensitivity to AVP in modifying the behavioral and cardiac response to mild unexpected stress.

AVP caused a reduction in heart rate in all age groups already before the sudden reduction of background noise. This may be the result of a baroreceptor reflex-induced vagal activation. That this alternative is unlikely is suggested by our former study (29). Sixty min after peripheral administration of 10 μg/kg AVP a moderate bradycardia was found in rest conditions together with a slightly decreased blood pressure. Lebrun et al. (24) also showed that the systolic pressor response to 6 μg/kg AVP disappears within 60 min after administration. Therefore,the reduction of heart rate may be caused by a direct action of

vasopressin on the heart (14,35), through effects of the peptide on coronary blood flow and oxygen availability within the myocardium (2,27).

In addition to the general reduction in heart rate, AVP extended the bradycardiac stress response in 4 and 14 months old animals. These data support the finding of an effect of AVP on acute cardiac stress responses in young rats as described previously (20).

Administration of AVP enhanced the magnitude of the behavioral immobility response to stress in 14 months old animals, whereas it decreased this response in 25 months old rats. This differential age-related effect of AVP on the behavioral immobility responses is reflected in the significant interaction between age and treatment. Peptide treatment further prolonged the duration of immobility behavior in 4 months old animals.

Both centrally (10) and peripherally (25) located mechanisms are hypothesized to be involved in vasopressinergic modulation of stress-related autonomic and behavioral responses. The extension of the bradycardiac stress responses may reflect a sensitization of baroreflex control of circulation (16). No data are available, however, on age-related changes in the vasopressinergic modulation of these reflexes. In numerous studies changes of vasopressinergic innervation patterns in the brain of aged rodents was shown (for review see 36). Age-related changes in vasopressin cells in the suprachiasmatic nucleus were reported (34). Dorsa and Bottemiller (12) found decreased AVP concentrations in a number of intra- and extrahypothalamic areas in aged rats, -e.g. in septum, the vascular organ of the lamina terminalis and the locus coeruleus. A study of Fliers et al. (15) revealed that age-related reduction in vasopressinergic innervation occurs in many areas of the brain controlling behavioral and autonomic responses. Only a few studies have been devoted to the effects of exogenous AVP in behavioral tasks in aged animals. While the present study addresses the effects of AVP on attentional processes, most of these studies involve age-related effects of AVP on memory measures. In late-adult rats (13-14 months old) AVP enhanced cardiac and behavioral stress responses (5) and improved memory function (37). Cooper et al. (9) showed that AVP facilitated the conditioned taste aversion in 19 and 24 months old rats.

While the present findings in the younger groups confirm the reported effects of AVP on behavioral and autonomic performance (5,20,25) the failure of the peptide to elicit similar effects in aged rats indicate a decreased sensitivity to AVP in these old animals. A cause of the vasopressinergic hyposensitivity in aged rats may be a decreased sensitivity or number of vasopressinergic receptors during aging. No information to our knowledge is available yet about age-related alterations in the properties of vasopressinergic receptors in the brain. Peripherally, however, a strong decrease in vasopressin binding was found in the kidney (33) and the liver (26) of aged rats. Administration of AVP failed to correct the impaired urine concentration in aged rats (1), indicating the decreased sensitivity of the aged kidney to the antidiuretic effect of AVP. However, vascular smooth muscles of old rats show an increased sensitivity to AVP (17). Accordingly, there are probably organ-specific differences in the sensitivity to vasopressin during aging, -i.e. age does not affect all vasopressinergic receptors uniformly. A third possible mechanism of the reduced effect of AVP in aged rats might be related to age-dependent changes in receptor-stimulated second-

messenger activation (38). There is extensive evidence that central and peripheral catecholaminergic systems interact with neuropeptides such as AVP in the modulation of behavior (6,23,32). Since peripherally applied d-amphetamine enhances the bradycardiac stress response in aged rats (28), it is also possible that AVP indirectly modulates the cardiodeceleratory response to emotional stress through activation of aminergic systems in the brain. The failure of AVP to restore the bradycardia in old animals therefore can be related to the reported age-related reduced activation of central catecholaminergic systems (39).

Whether the effects of systemically applied AVP in affecting cognitive processes are peripherally or centrally mediated is still an issue of major discussion (11,22). Whereas De Wied and colleges (10) suggested a direct central action of vasopressin, Le Moal et al. (25) hypothesized that hemodynamic responses shortly after the peptide injection were causing the behavioral effects of AVP. Koob et al. (21) suggested that systemically and centrally administered AVP can influence behavior in a homologous manner but by different mechanisms of action. Our data fit with the decreased peripheral sensitivity, but a central direct action can not be excluded.

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Chapter 8

BEHAVIORAL AND NEUROENDOCRINE EFFECTS OF VASOPRESSIN IN RESTING AND STRESS CONDITIONS

B. Buwalda, C. Nyakas, J.M. Koolhaas and B. Bohus

ABSTRACT

In order to understand the mechanisms by which systemically administered arginine-vasopressin (AVP) modulates behavior and autonomic functioning, (neuro)endocrine and behavioral measurements were taken in young adult male Wistar rats. The effects of subcutaneously administered AVP (6 μg/kg b.wt.) were determined in resting and mild emotional stress conditions before and 5 to 92 min after treatment.

Systemic administration of AVP caused a biphasic increase in blood glucose level, a long-lasting increase in CORT secretion, and a decrease in circulating NE under resting condition. It did not affect adrenal medullary E secretion. The stress induced sympathetic activation as reflected in plasma NE level was inhibited 60 min after AVP administration. In resting condition AVP caused a 60 min lasting increase in grooming behavior with a concomitant decrease in time spent resting. Sixty min after administration, AVP-treated rats were less active after the mild emotional stress of transportation and placement in a novel environment than vehicle-treated control rats.

The results suggest that AVP may modulate behavior not only by its hypothesized direct action in the brain or by its systemic pressor effect, but also by enhancing blood glucose and adrenal CORT secretion. The vasopressinergic sympatho-inhibitory action may play a role in the previously reported vasopressinergic enhancement of parasympathetic cardiac stress responsiveness. Furthermore, this action of AVP and the suppression of stress induced active behavior suggest that AVP may also be a neuroendocrine factor with dearousing properties.

INTRODUCTION

Apart from its classically described antidiuretic and vasopressor action the neurohypophyseal peptide arginine-8-vasopressin (AVP) produces various behavioral changes. It prolongs extinction and improves consolidation and retrieval processes in avoidance learning tasks (5,16,24,39,50), and modifies behavioral responses to novelty stress (8,47,52). Previous research in this laboratory showed that AVP also serves as an important modulator of a vagally mediated bradycardiac response to an emotional stressor (2,4,8,25).

As to the mechanisms by which AVP exerts its behavioral (cognitive) actions there is ambiguity in the literature. De Wied and colleagues (16,17) hypothesized that the modulatory effect of AVP on memory processes is predominantly exerted through direct actions in the brain. Others have challenged this view suggesting that peripheral effects of AVP leading to increased arousal are responsible for the behavioral effects of the peptide (19,39). Based on observations that the cognitive (mnemonic) behavioral actions of systemically applied AVP could be reversed by antagonizing the pressor effect of AVP, the arousal changes secondary to increases in blood pressure were assigned as responsible mechanisms for the behavioral action of AVP (39). Since application of the vasopressin analogue desglycinamide-AVP, which lacks virtually all pressor activity (16), and AVP administered directly into the brain in doses that do not enhance systemic blood pressure also clearly elicited behavioral effects (16,33,36), it was hypothesized that systemically and

centrally administered AVP can influence behavior in a homologous manner but by different mechanisms of action (33,35).

Since it is not necessary to produce peripheral vasoconstriction to obtain the behavioral effects of AVP, vasopressin may modulate behavior not only by a direct vasopressinergic action in the brain but alternatively also by eliciting peripheral (neuro)endocrine changes via central or peripheral mechanisms. AVP affects sympathoadrenal (37) and adrenocortical activity (9,20,41). Therefore changes in these adrenal hormones may also play a role in the behavioral effects of AVP. The adrenal hormones corticosterone (CORT) (15), and epinephrine (E) (7,22,27,43) are known to play an important role in regulating cognitive processes. Changes in blood glucose represent another memory enhancing humoral factor (21,54).

In order to understand the dynamics and the mechanisms by which AVP modulates behavior, the effects of systemically applied AVP on the level of blood glucose and plasma levels of the catecholamines E and NE, and CORT in resting and mild emotional stress conditions were investigated. Observed effects of AVP on sympathetic activity may also yield information on the mechanisms contributing to the enhanced vagal stress-responses after administration of AVP. Behavior was analyzed to study whether systemically administered AVP alters the emotional "state" in animals during these conditions.

METHODS

Animals and housing

Male Wistar rats (4 months old), originating from the Winkelman substrain, were housed individually in clear Plexiglas cages (25x25x30 cm), with food and water ad libitum, on a 12h light-dark regime (light on between 07.30h -19.30h) at a room temperature of 21 ±2°C.

Surgery

To study the effects of AVP administration on blood glucose levels, plasma catecholamines and CORT, half of the animals (n=12) were provided with a permanent silicon catheter (0.95 mm OD., 0.50 mm ID.) in the right atrium. The catheter was inserted via the right jugular vein and externalized on the top of the skull according to the techniques described earlier (49). These catheters allow frequent blood sampling in unrestrained and undisturbed freely moving rats (55). After surgery, the rats were allowed to recover for one week before the start of the experiments. During this week animals were handled and connected to the blood sampling tubing twice to habituate to the sampling procedure.

Blood sampling procedure

Blood samples of 0.45 ml were taken at each sampling point. After each sample the same quantity of heparinized (25 units per ml) donor blood was given to minimize the changes in blood volume with related changes in hemodynamics (49).

Experimental procedure

Neuroendocrine effects of AVP.

All experiments were performed between 09.00 and 13.30 hr, -i.e. in the period of stable and low plasma levels of E, NE and CORT (13). Six rats were injected subcutaneously (SC) with AVP. Saline (SAL) was administered to 6 other animals as a control treatment. At least one hour before the start of the experiment the animals were connected to the sampling tubing in order to obtain reliable, stress-free basal values. Blood samples were taken in the homecage before and after AVP or SAL administration (at t=0 min). Basal samples were taken at t=-11 and t=-1 min. Neuroendocrine and glucose response measures were taken at t=5; 10; 20; 40; 60; 65; 70; 77; and 92 min.

The effects of AVP on endocrine responses to the mild stress of environmental change were studied one week later in the same animals. Basal blood samples were taken at -11 and -1 min. At t=0 min SAL or AVP was administered. Stress was presented one hour after administration of the peptide. At t=60 min the homecage of each rat was transferred to another, similar room where a constant background "white" noise (65 dB, 2-8 kHz) was produced by a noise generator. During the transfer, which lasted for about 2 min, rats were not handled. Stress values were sampled at t=65; 70; 77; and 92 min. A delay of one hour before stress presentation was chosen to interpret the results in comparison with studies that have considered the effects of SC applied AVP on retention or autonomic functioning. In these studies the peptide was often administered ±1 hr prior to the test (2,8). It has further been shown that the pressor effect of AVP in the dosage as used returned to within the normal range after 1 hour (38,40).

Behavioral effects of AVP.

In the other group of rats (n=12) behavioral effects of systemically applied AVP were studied in resting and stress conditions. The experiment was started when all animals were at rest, -i.e. with high scores for resting behavior and low scores for activity and grooming behavior. After administration of the vehicle or AVP, behavior was sampled in every 10 sec. The observer was not familiar with the treatment schedule. After a preliminary analysis 8 separately recorded behavioral elements were pooled in three categories. Sniffing with head movements, walking and rearing were considered to represent active behavior. Face and fur washing, scratching and genital grooming were considered to be grooming behavior. Sitting with eyes open or eyes closed, and sleeping were considered as resting behavior, because the majority of behavioral samples in this category was classified as sleeping behavior. Sixty behavioral scores were added in blocks of 10 min.

Treatment

Arginine-8-vasopressin (AVP) was dissolved in saline and injected subcutaneously (SC) in a dose of 6 μg/kg b.wt. at t=0 min. Saline (SAL) injections (1 ml/kg b.wt.) served as vehicle. The selection of the single dose of the peptide was based on both the results of previous studies of young and aged rats' cardiac response to emotional stress (4,8) and on several other studies indicating well defined behavioral effects of AVP (19,34,38,39). EEG recordings (18) further indicated a similarity between this SC dose and a "behaviorally relevant" intracerebroventricular dose of 1 ng. The rats were injected in a cross-over design with SAL or AVP. A 7-day wash-out period was allowed between injections in resting and stress conditions to minimize interactions of treatments.

Chemical determinations.

Blood samples of 0.45 ml were withdrawn for determination of plasma epinephrine (E), norepinephrine (NE) and corticosterone (CORT) concentration. The samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.01 % EDTA as antioxidant and 10 μl heparin solution (500 IU/ml) as anticoagulant. Blood was centrifuged at 4°C for 10 min at 5000 rpm, and 100 μl of the supernatant were stored at -20°C for corticosterone and at -80°C for the catecholamine measurements. Plasma CORT was measured by means of reversed phase high performance liquid chromatography, as described earlier (12). Determination of plasma catecholamine concentrations was performed by HPLC in combination with electrochemical detection (ECD) as described earlier (48), with minor modifications. The absolute detection limit for catecholamines is 0.5 pg per injection with a signal to noise ratio of 2.

Statistics

Results are presented as means ± SEM. Data were analyzed using a multivariate analysis of variance with repeated measures (MANOVA) followed by a two-tailed Student's t-test, or a Mann-Whitney U-test. Since stress responses were measured 60-90 min after administration of the substances, MANOVA testing was divided in analyzing the first 60 min and the last 30 min (60-90 min). A probability level of $p<0.05$ was taken as statistical significance for all tests.

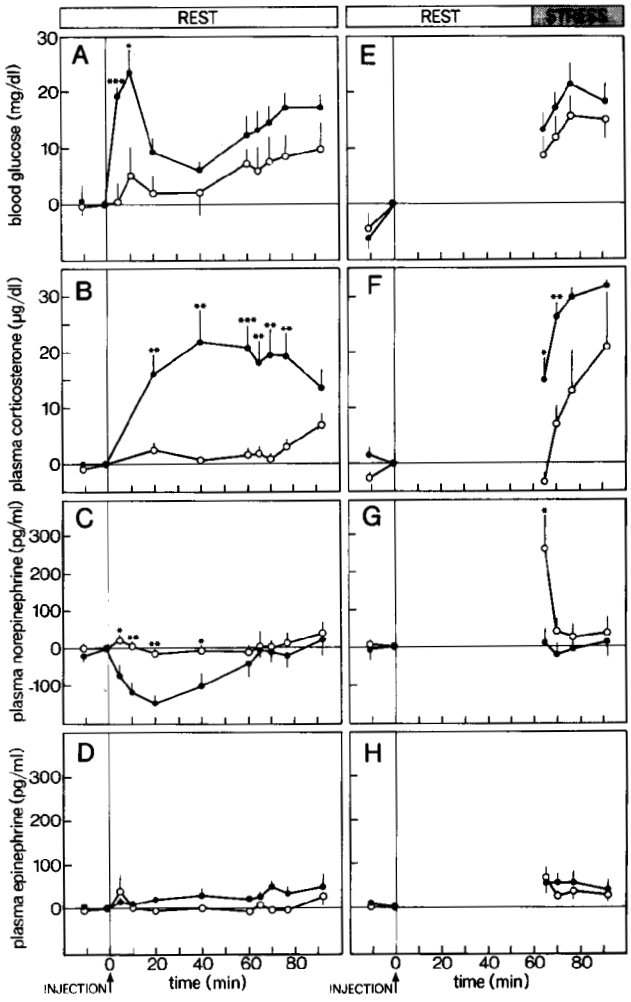

Fig.1

*In the left panel the effects of SAL (o-o) or AVP (●-●) administration on mean increases/decreases in blood glucose level (1A), plasma CORT (1B), NE (1C), and E (1D) levels in resting condition are shown. The right panel shows the effects of transfer of the home cage to a novel environment on alterations in glucose (1E), CORT (1F), NE (1G), and E (1H) 60 min after administration of SAL or AVP. The time of injection is indicated with an arrow. Means ± SEM of 6 rats are shown. *p<0.05; **p<0.01; ***p<0.001, determined by MANOVA and a two-tailed t-test.*

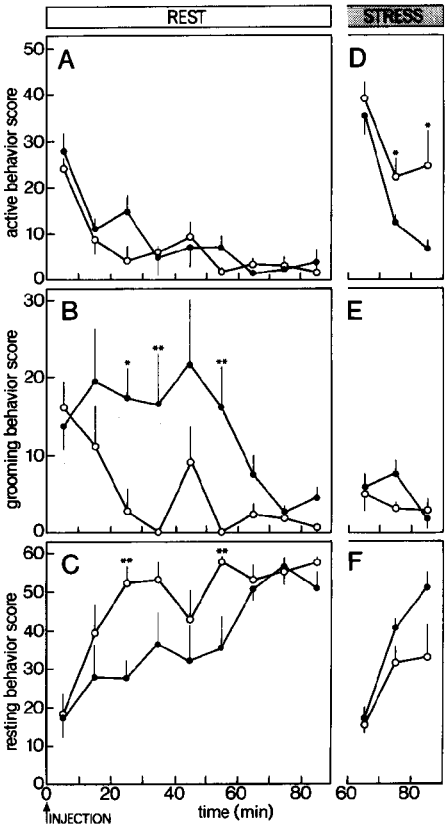

Fig.2

*In the left panel the effects of SAL (o-o) or AVP (●-●) administration on mean active (2A), grooming (2B), and resting behavior (2C) in resting condition are shown. The right panel shows one hour after administration the effects of AVP or SAL on stress induced behavioral changes in activity (2D), grooming (2E), and resting behavior (2F). Behavior was recorded every 10 sec. Since behavior was pooled in blocks of 10 min, each time point includes 60 behavioral recordings per rat. Means ± SEM of 6 rats are shown. *p<0.05; **p<0.01, determined by MANOVA and Mann-Whitney U-test.*

RESULTS

Neuroendocrine effects of AVP

Resting condition.

Blood glucose. Basal blood glucose levels were equal in SAL- (92±4 mg/dl) and AVP-treated (86±2 mg/dl) animals. During the first 40 min AVP administration caused a strong elevation of blood glucose, with a peak level (23±4 mg/dl) at t=10 min (Fig. 1A). MANOVA indicated a significant treatment effect over the first 60 min after AVP administration, $F(1,10)=9.09$; $p=0.01$, and an interaction of treatment with time, $F(4,40)=7.78$; $p=0.0002$. However, in SAL- and AVP-treated rats blood glucose increased in a similar way after t=40 min in comparison to pretreatment values ($p=0.01$). A treatment effect was therefore absent during this period.

Plasma corticosterone. Basal CORT level was 1.8±1.2 μg/dl in SAL-treated rats, and 1.0±0.7 μg/dl in AVP-treated animals. Figure 1B shows that AVP administration caused a long lasting increase in CORT secretion. During the first 60 min a treatment effect was present, $F(1,8)=17.79$; $p=0.003$, as well as a significant interaction of treatment with time, $F(3,24)=13.37$; $p=0.0001$. A peak level (21.8±5.6 μg/dl) was reached at t=20 min. The last 30 min a treatment effect was still present, $F(1,9)=39.16$; $p=0.0003$, although CORT level in SAL treated rats increased during this period in comparison to pretreatment values, $F(4,20)=4.52$; $p=0.009$.

Plasma norepinephrine. Basal levels of plasma NE were similar in SAL- (182±25 pg/ml) and AVP-treated (156±22 pg/ml) rats. Figure 1C shows that AVP caused a strong decrease in plasma NE level. This effect was significant during the first hour resulting in a treatment effect, $F(1,8)=10.38$; $p=0.01$, and a significant interaction of treatment with time, $F(4,32)=5.53$; $p=0.002$. The lowest NE level was reached 20 min after AVP was administered (-150±17 pg/ml). The last 30 min NE levels in AVP treated rats were back to baseline.

Plasma epinephrine. Basal plasma E level was 4.5±2.8 pg/ml in SAL-, and 4.5±4.5 pg/ml in AVP-administered rats. Neither AVP nor SAL treatment did cause a change in plasma level of E (Fig. 1D).

Stress condition

Blood glucose. Transportation of the home cage to another environment 60 min after administration of SAL or AVP caused in both SAL-and AVP-treated animals a significant

($p<0.0001$) increase in blood glucose, with peak levels reached at the 15th min ($t=77$ min) in the novel environment (Fig. 1E). AVP treatment per se failed to affect the glucose response to stress.

Plasma corticosterone. Stress of transfer caused an increase in plasma CORT in both SAL-, $F(4.8)=5.59$; $p=0.02$, and AVP-treated rats, $F(4,8)=46.7$; $p=0.0001$, as indicated in figure 1F. Although a treatment effect was present, $F(1,4)=8.43$; $p=0.04$, it was probably caused by the higher initial CORT level on $t=60$ min after AVP administration. The absence of a significant interaction between treatment and time, after transportation, indicates that AVP failed to alter the stress induced CORT response. Peak levels in both SAL- (20.7 ± 9.6 μg/dl) and AVP-administered (31.4 ± 1 μg/dl) rats were reached at $t=92$ min.

Plasma norepinephrine. One hour after the application of the vehicle or AVP, transportation stress caused a sharp and brief increase in plasma NE in SAL- (263 ± 92 pg/ml) but not in AVP-treated animals (Fig. 1G), resulting in a significant interaction between treatment and time, $F(3,30)=4.52$; $p=0.01$. Five min later plasma NE in vehicle-treated animals was back to baseline level.

Plasma epinephrine. Figure 1H shows that the transportation stress caused a very small increase in E in both groups of animals ($p=0.04$). Peak levels were 69.3 ± 25.3 pg/ml in SAL- and 56.2 ± 27.8 pg/ml in AVP-treated rats. Vasopressin did not elicit a treatment effect on E secretion.

Behavioral effects of AVP

Resting condition

Active behavior. Figure 2A shows that injection per se resulted in an increase in active behavior that was similar in SAL- and AVP-treated animals. The reduction of activity in time was also similar after both treatments.

Grooming behavior. The injection procedure caused some initial grooming behavior in both vehicle and AVP treated animals (Fig. 2B). This behavior diminished during the first 30 min in SAL-treated rats. Grooming behavior remained elevated in AVP-administered animals until $t=60$ min, resulting in a significant treatment effect over the first hour, $F(1,10)=11.47$; $p=0.009$. The last 30 min the vasopressin treatment effect was absent. No interaction between treatment and time was observed.

Resting behavior. During the first 10 min the amount of time spent resting was relatively low (Fig. 2C). Subsequently, resting behavior increased up to near peak level during the first 30

min in SAL-treated rats. This coincided a decrease in grooming behavior. The increase in resting behavior exhibited after AVP administration was much less and remained below that of the vehicle-treated rats up to sixty min. After 60 min the major behavioral components in both groups were equal. MANOVA over the first hour shows a treatment effect, $F(1,10)=11.47$; $p=0.009$. The treatment effect of AVP was absent during t=60-92 min.

Stress condition

Active behavior. Figure 2D shows that transportation to a novel environment 60 min after administration of SAL or AVP caused an initial increase in active behavior that was similar in both groups. Active behavior after AVP administration, however, progressively decreased, while activity in vehicle-treated animals remained elevated during the 30 min observation period. This is reflected in a vasopressinergic treatment effect, $F(1,10)=7.14$; $p=0.02$.

Grooming behavior. Transfer stress did not elicit substantial grooming behavior (Fig. 2E) when compared to the same period in resting conditions. MANOVA failed to show a treatment effect either.

Resting behavior. Figure 2F shows that both groups spent the same amount of time resting during the first 10 min after home cage transfer. In AVP-treated rats, however, the time spent resting progressively increased, while this increase was less in vehicle-treated animals. Statistical analysis yielded a treatment effect that was close to significance, $F(1,10)=4.2$; $p=0.06$.

DISCUSSION

The main findings were that peripherally administered AVP has differential neuroendocrine and behavioral effects, both during resting and mild stress conditions. Administration of AVP caused an increase in blood glucose level and CORT secretion, and markedly depressed NE level during resting conditions. It did not affect adrenal medullary E secretion. AVP caused a 60 min lasting increase in grooming behavior with a concomitant decrease in time spent resting during this period. Sixty min after administration the increase in active behavior caused by the mild emotional stress of transportation and placement in a novel environment extinguished more rapidly in AVP- than in vehicle-treated control rats. The stress induced sympathetic activation was inhibited one hour after AVP administration as indicated by the absence of a plasma NE response.

The observed physiological and behavioral effects of AVP during resting condition

are particularly apparent within the first hour after systemic injection, similar to previously reported pressor effects caused by this dose of AVP (38). Some of the effects observed after AVP administration may be indirectly caused by changes in one of the other presented variables.

During resting conditions AVP caused a sharp initial increase in blood glucose followed by a second and slower increase in blood glucose in both SAL- and AVP-treated rats. Since plasma NE decreased and E remained stable after AVP administration, the first rise in glucose is probably caused by the direct action of AVP on hepatic glycogenolysis (51). The second phase in the glucose enhancement after AVP administration cannot be easily explained because it is also present in the controls.

The stimulating effect of AVP on the secretion of adrenocorticotropin (ACTH), inducing adrenal CORT secretion is well known (9,20,41). The stress induced CORT response, however, was not potentiated by AVP pre-treatment, although CORT level at the onset of the stress was much higher.

AVP diminished plasma NE levels during resting conditions. This probably is caused by the inhibitory action of circulating AVP on sympathetic preganglionic neurons (26,28,32,37), although an increased utilization or degradation rate of NE after AVP cannot be excluded. Since sympathetic ganglia are protected by a blood-nerve barrier (46), a direct inhibitory action of systemically administered AVP on sympathetic preganglionic neurons implies transportation of AVP over this barrier. The ganglionic sympatho-inhibitory vasopressinergic effect does not exclude additional central actions of systemically applied AVP. It has been reported that circulating AVP acts at the level of the area postrema and locus coeruleus by altering noradrenergic function, and enhances in this way the inhibitory influence on the sympathetic system (45,53). One hour after AVP administration, plasma NE returned to the baseline level. Stress, however, at that moment failed to enhance sympathetic activity in AVP-treated animals, indicating that the inhibition of sympathetic activation is still present.

The finding that AVP fails to affect adrenal medullary E secretion in both resting and stress conditions supports the results obtained by Kvetnansky et al. (37).

Some of the endocrine and metabolic alterations caused by AVP may play a role in the behavioral properties of systemically administered AVP. Increases in blood glucose level similar in magnitude to the observed AVP-induced rise in glucose, have been associated with enhanced memory performance (21,54). The glucose enhancing effect shortly after administration of AVP may therefore play a role in earlier reported posttrial learning modulating properties of systemically administered AVP (17,24). Whether the enhancement of CORT secretion by AVP plays a role in the vasopressinergic modulation of cognitive functioning is not clear yet. The effects of CORT and AVP on extinction of inhibitory avoidance are even opposite (3,5). Postlearning administration of CORT facilitates, however, the consolidation of acquired immobility in the Porsolt swimming test (29). CORT further was shown to reduce the efficacy of E to consolidate a passive avoidance response (14). Since AVP and CORT are reported to have a common central site of action in the

hippocampal norepinephrine-stimulation of cyclic AMP formation (42), an interaction between AVP and CORT actions, however, cannot be excluded.

Previous studies showed that memory enhancing actions of AVP are absent in adrenomedullectomized rats (6). These and other data of Borrell et al. (7) indicated that AVP requires the presence of peripheral E in order to enhance memory function. The present study shows that spontaneous behavior during resting and stress conditions are affected by AVP in the absence of a detectable E response, suggesting that the AVP action on behavior is not mediated via an increased E release. Although less potent then E, systemic injections of NE can enhance retention in rats (23). The vasopressinergic inhibition of sympathetic activity as observed in this study suggest that cognitive effects of the peptide cannot be explained by changes in the levels of plasma NE.

Several observations in this laboratory demonstrated the vasopressinergic enhancement of parasympathetic cardiac stress responses (2,8,25). The inhibitory action of AVP on stress induced sympathetic activation may be of major importance in the potentiating effect of AVP on the vagally mediated bradycardiac response to stressors evoking behavioral immobility (2,8,25). During stressors of this nature sympathetic and parasympathetic outflow are activated in parallel. Together with an increased vagal activity following systemical application of AVP (11), the vasopressinergic blockade of stress induced sympathetic activity may cause the autonomic balance to shift towards the parasympathetic side, leading to a dominant vagal action on the heart.

In resting condition, the elevation of grooming behavior and the associated decrease in time spent resting appeared as major behavioral effects of AVP. These changes diminished one hour after peptide treatment. Induction of grooming behavior immediately after ICV administration of AVP has been reported earlier (44). The early post-treatment period of excessive grooming as caused by AVP coincided multiple physiological and endocrine changes. Excessive grooming may represent a displacement activity during an increased arousal state and serve restoration of the homeostatic status (10,30).

A remarkable effect of AVP is the rapid termination of the stress-induced behavioral activation. This effect of the peptide was visible after the disappearance of the intrinsic behavioral actions of AVP. Since glucocorticoids are implicated as modulators of extinction of stress-related behavior (1), the long-lasting elevation of CORT level in AVP-treated rats may play a role in this accelerated "dearousal".

Stress is often associated with increased grooming behavior (31). After the mild stress of transfer of the home cage, grooming behavior was not elevated when compared to the same period under resting conditions. The avoidance of handling the animals is probably responsible for this very low grooming activity.

Together the state- and time dependent modulation of spontaneous behavior suggest that AVP has arousing properties shortly after administration when marked pressor (38) and endocrine vasopressinergic effects are apparent. In a later phase AVP may facilitate dearousal mechanisms following stress-induced behavioral activation.

The neuroendocrine changes indicate that AVP may modulate cognitive functioning

not only by its hypothesized direct action on the brain or by its systemic pressor effect, but also by enhancing the level of blood glucose and adrenal CORT secretion.

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Chapter 9

CONCLUDING REMARKS

The studies presented in this thesis were designed to investigate whether the decline in parasympathetic responsiveness to stress in aged rats can be generalized for various (environmental) challenges. In addition, the contribution of this reduced vagal responsiveness in aged animals to autonomic dysbalance was determined. Finally, considerable attention was paid to the role of aminergic and vasopressinergic systems in the age-related reduction of vagal responses.

The results show that in aged rats parasympathetic responsiveness to a variety of challenges is reduced. However, sympathetic responsiveness is reduced as well. Therefore, autonomic output remains in balance during mild emotional stress situations. Enhancement of the aminergic "state" reinstates the vagal responsiveness to emotional but not to metabolic challenges in aged rats. Systemic administration of vasopressin improves and prolongs both the behavioral and vagal emotional stress responses in rats but only until late-adult age (14 months) is reached.

9.1.1. Adaptation to age-related functional decline through changes in behavioral, neuroendocrine, and autonomic responses.

Maintenance of homeostasis involves a complex series of integrative functions in which almost all body organs and systems participate. The classical "emergency" concept of Cannon (13) suggests that maintenance of homeostasis may be the result of interactions between brain, behavior, and the endocrine system, particularly of the adrenomedullary system. Selye's stress theory (40) emphasizes the importance of the pituitary-adrenocortical system in adaptation to environmental demands. A novel, behavioral physiological stress concept was presented by Bohus et al. (7) extending the concept to interactions between environment, behavioral characteristics and physiology.

It is assumed that the diseases that are generally associated with the degeneration caused by the aging process occur more frequently at senescence because the regulatory range of homeostatic mechanisms becomes restricted. Abundant evidence in aging studies, however, suggests that even though this range is diminished, homeostatic mechanisms compensate for the intrinsic functional decline in aged organisms at peripheral organ and central nervous system level (see Fig.1). Compensation for or adaptation to an age-related functional decline can also occur through changes in the behavioral, neuroendocrine, and

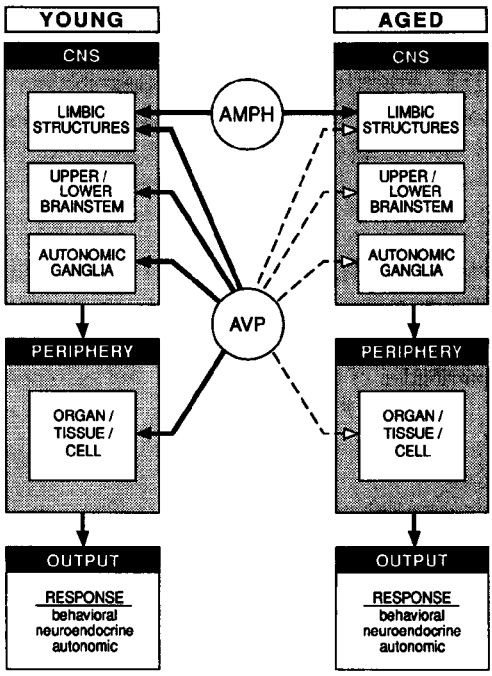

Fig.1. *Different organizational levels at which adaptation to age-related functional decline may occur. Furthermore, the levels via which systemically applied AMPH or AVP may enhance the parasympathetic and behavioral responsiveness are indicated by arrows. The dotted AVP arrows represent the reduced vasopressinergic modulation of behavioral and parasympathetic responses in aged rats.*

autonomic stress-responses that organize the complex physiological and behavioral adjustments of the organism to various challenges.

In this thesis it is shown that sympathetic discharge as reflected in circulating levels of NE as well as parasympathetic responsiveness as measured by changes in heart rhythm to both conditioned (25) and acute emotional stressors (chapter 5) are diminished in aged rats. By means of a decreased responsiveness in the two branches of the autonomic nervous system, homeostasis appears to be maintained. Other reports addressing age-related changes in cardiovascular and neuroendocrine responsiveness to stress (14,31) suggest an impaired homeostatic regulation of the sympathetic nervous system during stress. This suggestion, however, is largely based on results obtained after very severe stressors like cold water immersion, long-lasting immobilization, and cellular glucoprivation. The functional significance in every day life of this failing response in aged animals to these severe types of stress can be questioned, however. More likely, these experiments demonstrate the reduction in the maximal regulatory range of homeostatic mechanisms.

Animals also compensate for the intrinsic functional decline during aging by adapting their behavioral response to challenges. Much research considering behavioral and physiological stress responses in aging has been performed in experiments in which animals fail the ability to control or to cope with the stressor (see 43). In most real life stress

situations, individuals can cope with stressors by adopting an active or passive behavioral strategy (7). Old individuals adopt a behavioral response that incorporates all learned skills and past experiences, possibly leading to an increased avoidance behavior (34). In this way the behavioral response to stress can compensate for the age-related functional decline in the regulatory range of physiological stress mechanisms.

The neuroendocrine CORT response in aged rats to mild, acute stress (chapter 5) was similar to the one observed in young rats. The results obtained in conditioned stress situations (25) support this finding. However, an increase in the basal level of circulating CORT was found. One may question to what extent the increased basal levels of circulating CORT represent unstressed, resting levels. The experiment considering acute stress responses was followed by a study in which conditioned stress responses were studied in the same rats (25). Basal CORT level in aged rats one week after the exposure to mild stress did no longer differ significantly from that in young ones because of a relative fall of basal CORT levels in the aged rats. One day after an inescapable footshock, however, basal CORT level in aged rats appeared to be elevated in comparison to young rats that was back to pre-shock level. Elevated basal levels of CORT in aged rats may therefore reflect the slow recovery of adrenocortical activation to any kind of challenge whether being experimentally planned or incidental. Whether the delayed recovery of adrenocortical activation in aging is detrimental (39), or possibly an adaptational, homeostatic process that occurs in aging needs further research.

9.1.2. Adaptation of responses to age-related functional decline through changes at different organizational levels.

The functional decline in regulatory range and maintenance of homeostasis must be reflected at different organizational levels of the behavioral and physiological response. Age-related alterations in autonomic functioning is studied frequently at the isolated tissue level. Numerous changes with increasing age have been reported in the number or affinity of receptors and in postreceptor effector mechanisms (19). Since corresponding alterations (distortions) cannot be obtained in in vivo stress responses, the reported alterations at tissue level were suggested (section 1.2.1) to be largely matched by compensatory mechanisms at higher organizational levels. The age-related alterations observed at cellular level possibly do not represent pathological distortions but rather homeostatic processes.

Considering cardiovascular responsiveness many age-related changes have been reported both at specific peripheral organ level (28) and at neural system level (11, this thesis). Many brain regions including the brainstem, midbrain and forebrain (Fig.1) are closely involved in the control of the cardiovascular system (10). Systems mediating tonic control and reflex changes are principally located in the lower brainstem (4), whereas upper brainstem, hypothalamic, and limbic forebrain systems are largely responsible for the integration of sensory information from viscera and environment with the neural and humoral factors controlling cardiovascular functions (30,37). The age-related alterations in the systems involved in cardiovascular functions at all these levels (section 1.4), may partly

reflect compensatory mechanisms.

The reduction of the preabsorptive insulin response (PIR) in aged rats (chapter 4) did not result in a failing glucose homeostasis during the first 5 min after food intake. This may indicate that in aged rats also mechanisms are present that compensate for the diminished PIR. As described for the cardiovascular responsiveness, this compensation probably takes place at several levels. More insulin can be secreted by the increase in volume of pancreatic islets of Langerhans during aging (16). Carbohydrate absorption after food intake may also be delayed in aged rats. As for cardiovascular responsiveness additional compensation may be organized at higher levels. The interaction of NE and acetylcholine in the regulation of islet hormone secretion (1,12) may change with age. Furthermore, central nervous mechanisms are involved in the maintenance of metabolic homeostasis (5,27,38,41). Finally, also age-related changes at various levels of the brain may represent compensatory mechanisms for the intrinsic functional decline.

9.2. Aminergic and vasopressinergic modulation of parasympathetic and behavioral responsiveness.

Since central aminergic and vasopressinergic neural systems are closely involved in the regulation of behavioral and autonomic output, it was hypothesized that age-related changes in these systems are responsible for the reduction of the parasympathetic responsiveness. By changing the aminergic and vasopressinergic "state" in young and aged rats with systemically administered amphetamine (AMPH) or vasopressin (AVP) respectively, this hypothesis was tested. Because both the drug and the peptide were administered peripherally, physiological and behavioral adjustment of the organism to challenges may be influenced at all levels.

AMPH probably enhances vagal responsiveness (chapter 2 and 4) by enhancing central aminergic activity since a direct peripheral effect of AMPH would be expected to antagonize vagal responsiveness via its sympathomimetic actions. Since AMPH failed to alter basal heart rate, blood glucose or plasma insulin level (chapter 2, 3, and 4) this action is not simply due to a general aminergic activation in the brain. Rather, a modulation of the vagal responsiveness is likely. Activation of aminergic systems in hypothalamic and limbic brain structures innervating directly or indirectly the vagal output nuclei may be responsible for this type of vagal modulation. These higher brain systems are involved in the modulation and integration of sensory information from viscera and environment with cognitive information coming from limbic systems. Increasing aminergic activity in these structures may alter the appraisal of sensory information, and enhance in this way the response to challenges.

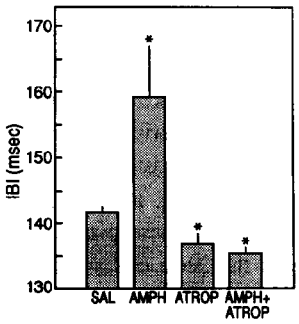

*Fig.2. Heart rate expressed as interbeat interval (IBI) of 24-mo-old rats during the first min of a conditioned stress of forced exposure to the compartment where a footshock was delivered earlier (see Ch.2). Thirty min prior to the test rats were injected (IP) with saline (SAL) or d-amphetamine sulfate (AMPH; 0.5 mg/kg). Other groups were injected 45 min prior to testing with atropine methyl nitrate (ATROP; 1 mg/kg), or ATROP followed by AMPH. *p<0.05 drug vs. SAL*

In some experiments attention was focussed on the mechanisms through which AMPH may enhance the behaviorally induced vagal responses (unpublished data). Blockade of the muscarinic receptors with atropine methyl-nitrate fully blocked the conditioned bradycardia enhancing effect of AMPH treatment in aged rats (Fig.2). Since atropine methyl-nitrate blocks peripheral muscarinic receptors only, it can be concluded that the increased stress-induced cardiac inhibition after AMPH administration is caused by an increased vagal action on the heart.

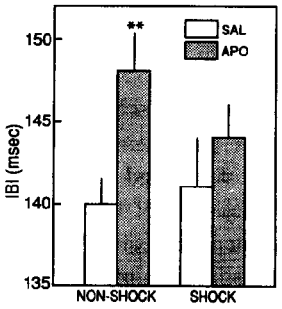

*Fig.3. Heart rate expressed as interbeat interval (IBI) of 24-mo-old rats during the first min of forced exposure to a compartment before and one day after an inescapable footshock was delivered. Thirty min prior to testing the rats were injected (IP) with saline (SAL) or apomorphine (APO). **p<0.01 APO vs. SAL.*

AMPH may act via enhanced release of central DA, NE, or 5-HT. The DA receptor agonist apomorphine (APO) was administered to aged rats in a similar experimental setup as used in the AMPH experiments in order to establish whether the enhancement of stress-induced bradycardia after AMPH treatment is mediated via dopamine (DA) release. Pretest APO administration to aged rats caused a reduction in heart rate during unstressed non-shock measurements but failed to restore the bradycardia stress-response (Fig.3). Therefore, the effect of AMPH on vagal stress responses (chapter 2 and 3) probably is mediated through central NE systems (see section 1.3.2). The role of 5-HT cannot be excluded (24).

AMPH also failed to reinstate the absent PIR in aged rats (chapter 4). Several factors were suggested to contribute to the differential effect of AMPH on the two aspects of parasympathetic responsiveness. An increased behavioral arousal by AMPH probably playing a permissive role in the stress evoked bradycardia being one of these factors. Alterations at the level of the pancreatic B-cell in the responsiveness to cholinergic stimulation as another possible factor, has been studied recently. Intravenously administered carbachol induces insulin secretion in young and aged rats. Peak increases in plasma insulin in young rats (44.8±2.6 μU/ml) were twice as high as in aged rats (20.8±4.8 μU/ml). These preliminary results indicate a decrease in the rapid insulin secreting capacity of the islets of Langerhans in aged rats or a decreased B-cell responsiveness to cholinergic stimulation, which may explain the failing effect of AMPH to reinstate the PIR in aged rats.

The results concerning the absence of effect of AVP on the behaviorally induced bradycardia in 26-27 months old rats (chapter 3) appear to be in contrast with earlier findings (8). AVP (3 and 10 μg/kg b.w.) reinstated the stress-induced conditioned bradycardia in 14 months old rats. It was suggested in chapter 3 that differences in age may underlie these conflicting results. The failure of AVP to reinstate the PIR in aged rats (chapter 4) may be due to cholinergic dysfunction, but an age-related vasopressinergic hyposensitivity is also a feasible alternative. Chapter 7 shows this development of hyposensitivity to AVP in aging rats. AVP failed to enhance the behavioral and cardiac response to mild unexpected stress in rats older that 14 months. These results are in agreement with a decrease in peripheral (renal) vasopressin binding in aged rats as reported by Miller and Dorsa (32) and Ravid et al. (36). Studies concerning age-related alterations in the properties of vasopressinergic receptors in the brain seem to us imperative to determine the central contribution to this phenomenon.

Vasopressin was hypothesized to be a neuroendocrine factor that principally serves passive coping activity through behavioral conservation/withdrawal and parasympathetic activation (8). Active coping animals in contrast are sympathetically dominated. Therefore, the reduced bradycardiac stress response in aged rats and the development of vasopressinergic hyposensitivity in aging could be consistent with active coping strategies by aged animals. The behavioral responses in aged rats, however, argue against a shift to active coping in aging. The more sluggish reaction of aged rats to environmental challenges is well documented. Behavioral immobility responses are not decreased in aged rats (chapter 2) and passive avoidance latency even increases in rats of 21 months old (34).

It is a matter of discussion (17,18,29) whether systemically applied AVP affects cognitive and autonomic processes through peripheral or central actions. In chapter 3, 4 and 7 it was suggested that some of the effects of peripherally administered AVP on heart rate and insulin secretion can be partly caused by direct effects at the level of the peripheral organ. AVP may suppress cardiac rate by effects of the peptide on coronary blood flow and oxygen availability within the myocardium (6,33). In chapter 4 it was suggested that the stimulation of pancreatic B-cell phosphoinositide second messenger metabolism by AVP may be involved in the enhancement of insulin secretion to food intake in young rats.

Another well-known effect of AVP at the peripheral organ level is its vasopressor effect (21). It has been hypothesized that this pressor effect is indirectly causing the behavioral (mnemonic) effects of systemically applied AVP (29). In order to avoid direct pressor effects, the vasopressinergic state in adult rats was manipulated by neonatal administration of low or high doses of AVP (chapter 6). This manipulation downregulates peripheral AVP receptors (20). The results indicated that an increased availability of vasopressin during the first week after birth markedly affects behavioral reactivity to novelty and adult memory processes. The adult bradycardiac response to emotional stress was much less affected by neonatal AVP treatment. Differences in the developmental phase of the vasopressinergic systems involved in the regulation of behavior and autonomic functioning may be responsible for the differential effect on behavioral and cardiac responses. Another explanation may be that direct pressor or endocrine effects of systemically applied AVP are of decisive importance in the enhancement of vagally mediated cardiac stress responses but not in the modulation of behavioral stress responses.

The role of systemically applied AVP in regulating autonomic stress responses is described in chapter 8. Since the modulation of behavioral and stress-induced bradycardia is organized at higher brain levels, AVP may act directly and/or indirectly on these higher brain structures (26). Besides acting at brainstem level (9), AVP may inhibit sympathetic responsiveness at the level of sympathetic ganglia (22). The sympathetic inhibition may be of importance in the vasopressinergic enhancement of vagal stress responses (chapter 8). In order to achieve a direct vasopressinergic effect on cholinergic transmission in the ganglia (22), the peptide has to be transported across the blood-nerve barrier. Although the possibility of transportation of AVP across the blood-brain barrier has been argued against (2), the results on sympathetic activity (chapter 8) indirectly suggest the possible transportation and central action of peripherally administered AVP.

9.3. Factors that reduce the regulatory range of homeostatic mechanisms in aging.

Whereas the maximum life span of humans appears to be constant in evolutionary studies of aging and longevity (15), the percent survival at intermediate ages is not. This possibly indicates that the range of homeostatic mechanisms is susceptible to various detrimental metabolic, physical, and psychic influences. With increasing chronological age, essentially all body functions in every organism are found to decline. Studies in humans show that persons that are unusually long lived and are able to live past 100 years do so not because they age more slowly but instead because they age more uniformly (23,35). That is, they appear not to suffer from any particular weak link in their bodily functions such as from heart disease or diabetes (15,23).

The study of parasympathetic and sympathetic autonomic responsiveness as presented in this thesis supports a hypothesis that a uniform functional decline serves the maintenance of homeostasis in aged rats. Various lifestyle patterns like overfeeding (see for review 3) and chronic stress, however, may influence this autonomic balance and thereby

threaten a uniform decline.

In a recent review Swaab (42) proposed that activation of nerve cells within the physiological range leads to maintenance of neurons in aging and Alzheimer's disease. This "use it or lose it" principle might present an additional factor that influences the speed in which the range of homeostatic mechanisms decreases in aging.

In this thesis parasympathetic responsiveness is shown to be reduced in aged rats. The results suggest that alterations of aminergic systems at higher brain levels are involved in this diminished vagal activation in aging, whereas age-related changes in vasopressinergic systems both at central and peripheral levels may play a role in this respect as well. When sympathetic responsiveness diminishes also, as was demonstrated in the experiments presented in this thesis, homeostasis seems to be maintained. However, numerous risk factors exist that threaten a uniform decline of physiological functions in aging. With an increasing number of elderly people in the developed nations of the world, the study of the impact of these risk factors on the maintenance of homeostasis and the speed in which the regulatory range of homeostatic mechanisms decreases will become of growing importance.

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SAMENVATTING

Mens en dier worden in hun dagelijks leven voortdurend blootgesteld aan fysiek en mentaal belastende gebeurtenissen. Om zich aan te passen aan deze zogenaamde stressoren kan een individu reageren met veranderingen in het gedrag. Verder probeert het lichaam het inwendige milieu constant te houden met behulp van autonome- en neuroendocriene processen.

De klassieke stresstheorieën besteden vooral aandacht aan de neuroendocriene activatie van het sympathicus-bijniermerg en het hypofyse-bijnierschorssysteem. Dit proefschrift richt zich vooral op de reactiviteit van het autonome zenuwstelsel. Het autonome zenuwstelsel verzorgt de regeling van de orgaanfuncties in het lichaam en bestaat uit twee anatomisch en functioneel gescheiden delen, de zg. sympathicus en parasympathicus. De invloed van de sympathicus en de parasympathicus op de organen is in veel gevallen tegengesteld. Dieronderzoek heeft aangetoond dat verschillen in de mate van beheersbaarheid van bedreigende situaties sterk bepalend zijn voor het autonome reactiepatroon. Wanneer door actief gedrag (bv. vechten of vluchten) de dreiging verminderd kan worden, gaat dit voornamelijk gepaard met een sympathische activatie. Deze sympathische activatie uit zich onder andere in een versnelling van de hartslagfrequentie en een verhoging van de bloeddruk. Er zijn echter ook situaties waarin wordt gekozen voor een passieve gedragsstrategie. In deze situaties probeert het dier door niet te bewegen een eventuele dreiging vermijden. Dit kan zich bijvoorbeeld voordoen als een dier geconfronteerd wordt met een onbeheersbare situatie of als een buitengewone alertheid wordt vereist. Tijdens dit immobiele gedrag wordt behalve het sympathische ook het parasympathische zenuwstelsel geactiveerd, hetgeen zich met name uit in een initiële verlaging van de hartslagfrequentie (bradycardie). Deze inleidende parasympathische component van de autonome stressrespons kan bijvoorbeeld worden gemeten in jonge ratten tijdens een aangeleerde angstreactie, d.w.z. de dieren verwachten een tijdens een trainingsperiode ontvangen aversieve electrische prikkel. Onderzoek voorafgaande aan de resultaten gepresenteerd in dit proefschrift liet zien dat oude ratten in deze situatie nog wel de gedragsmatige immobiliteitsrespons vertonen, maar dat de parasympathisch geïnduceerde hartslagfrequentiedaling sterk is afgenomen.

Dit proefschrift richt zich daarom eerst op de vraag in hoeverre tijdens veroudering de parasympathische reactiviteit meer in het algemeen is afgenomen.

Het centrale zenuwstelsel speelt een sleutelrol in de coördinatie van de gedrags- en autonome responsen die veroorzaakt worden door stressoren. Aminerge en vasopressinerge neurotransmittersystemen in de hersenen zijn in belangrijke mate betrokken bij de coördinatie van stressresponsen. Verder zijn er in de literatuur tal van aanwijzingen beschikbaar dat deze systemen tijdens het verouderingsproces in functioneren en morfologie aangetast worden. Dit proefschrift zal zich daarom tevens richten op de rol van aminerge en vasopressinerge neurotransmittersystemen in deze leeftijdsafhankelijke afname. Deze vraagstelling werd onderzocht in een diermodel waarbij de rat als proefdier werd gekozen.

De meeste experimenten werden uitgevoerd in jong volwassen (3 maanden oude) en 24 tot 27 maanden oude ratten. Als maat voor de parasympathische reactiviteit werd de hartslagfrequentiedaling tijdens emotionele stress en plasma insuline gehalte tijdens het eten van een maaltijd gemeten. Om sympathische en neuroendocriene activiteit te kunnen meten werd gebruik gemaakt van permanente hart- en/of aortacatheters. Hiermee kunnen kleine hoeveelheden bloed worden afgenomen en kan de bloeddruk worden gemeten zonder dat de ratten hiervan hinder ondervinden. Verder werd m.b.v. miniatuur FM-zenders het electrocardiogram (ECG) van de dieren geregistreerd.

Om de parasympathische reactiviteit in oude ratten te bestuderen, werden de dieren geconfronteerd met psychische stressoren die gedragsmatig immobiliteit veroorzaken. De dieren werden daartoe gedwongen blootgesteld aan een omgeving waar ze eerder een onvermijdbare onaangename prikkel ervaren hadden. In deze situatie is er sprake van een geconditioneerde angstverwachting waarbij cognitieve processen als leren en geheugen een belangrijke rol spelen. Daarnaast werden ratten geconfronteerd met een acute stress-situatie waarbij niet zo zeer de genoemde cognitieve processen van belang zijn, maar aandacht en attentie een grotere rol spelen. Bij deze test werden de dieren geplaatst in een zgn. open veld en werd een constant aanwezige, niet-aversieve achtergrondruis (65 dB) plotseling uitgeschakeld. Deze stress van onverwachte stilte veroorzaakt bij de proefdieren een zeer gespannen alertheid op eventuele verdere veranderingen in hun directe omgeving. Beide stressoren veroorzaken gedragsmatig immobiliteit die gepaard gaat met een daling in de hartslagfrequentie.

In de psychische stress-situaties spelen het gedrag en cognitieve processen een belangrijke rol. Om inzicht te krijgen in de parasympathische reactiviteit waarbij deze processen minder cruciaal zijn is ook gebruik gemaakt van een metabole stimulus. Tijdens het eten van een maaltijd wordt insuline in een bifasisch patroon afgegeven door de pancreas. Onmiddellijk na de start van de maaltijd wordt, voor er een stijging van het bloedglucose gehalte gemeten kan worden, insuline afgegeven. Deze insulinerespons wordt ook wel de pre-absorptieve insulinerespons (PIR) genoemd en wordt veroorzaakt door een activatie van de parasympathicus. Deze snelle, glucose onafhankelijke, insulineafgifte wordt gevolgd door een tweede glucose afhankelijke insulinerespons. In jonge en oude ratten is ook deze PIR gemeten.

De aminerge betrokkenheid bij deze parasympathische responsen werd onderzocht door onderhuids (subcutaan) amfetamine toe te dienen kort voor de uitvoering van de experimenten. Amfetamine is een psychostimulant en stimuleert de afgifte van catecholaminen op presynaptisch niveau. De vasopressinerge betrokkenheid werd bestudeerd door het hypofysehormoon arginine-8-vasopressine subcutaan toe te dienen.

De resultaten verkregen uit het onderzoek laten zien dat de parasympathische reactiviteit in oude ratten in de genoemde situaties afgenomen is (hoofdstuk 2,3,4). Verder blijkt amfetamine zowel de afgenomen geconditioneerde (hoofdstuk 2) als de acute emotionele stress geïnduceerde bradycardie respons te kunnen herstellen in oude ratten

(hoofdstuk 3). De verminderde parasympathische insulinerespons tijdens het eten wordt niet hersteld door amfetamine toediening (hoofdstuk 4). De resultaten verkregen na vasopressine toediening laten zien dat alhoewel de parasympathische respons in jonge ratten versterkt wordt, dit niet gebeurt in 26-27 maanden oude ratten (hoofdstuk 3,4). Dit was verrassend, omdat vooronderzoek in de geconditioneerde emotionele stress-situatie had laten zien dat de afwezige bradycardie respons in 14 maanden oude dieren wel hersteld werd na vasopressine toediening. Herhaling van het experiment met dieren in 4 leeftijdsgroepen laat zien dat tussen 14 en 20 maanden, ratten ongevoelig worden voor de vasopressinerge modulatie van gedrags- en autonome stressresponsen (hoofdstuk 7).

Om te onderzoeken of een verminderde parasympathische reactiviteit zou kunnen leiden tot een verstoorde autonome balans tijdens emotionele stress-situaties, is in vrijbewegende jonge en oude ratten naast de hartslagfrequentie ook de bloeddruk gemeten in geconditioneerde en niet-geconditioneerde stress-situaties. Tevens zijn bloedmonsters afgenomen om gehaltes van de plasma catecholaminen noradrenaline en adrenaline en het bijnierschorshormoon corticosteron te kunnen meten. In dit proefschrift zijn de resultaten gepresenteerd van de acute stressor (hoofdstuk 5). Samen met de elders gepubliceerde gegevens blijkt dat in oude dieren niet alleen de parasympathische, maar ook de sympathische reactiviteit afgenomen is. Uit deze resultaten kan geconcludeerd worden dat de autonome balans tijdens veroudering gehandhaafd lijkt te worden. Verder blijkt dat in oude ratten na stress de herstelfase voor de plasma catecholaminen vertraagd is en dat de basale corticosteron secretie verhoogd kan zijn.

Terwijl de resultaten na amfetamine toediening lijken te wijzen op een rol van centraal nerveuze aminerge systemen in de verminderde parasympathische reactiviteit in oude ratten, is het mechanisme waarmee vasopressine zijn modulerende werking op gedrags- en autonome responsen uitoefent, minder duidelijk. In de literatuur wordt veelvuldig gediscussieerd over de manier waarop vasopressine het gedrag beïnvloedt. Er zijn hypotheses waarin wordt uitgegaan van een direct centrale werking van perifeer toegediende vasopressine en er zijn aannames dat de werking van vasopressine op het centrale zenuwstelsel indirect verloopt, met name via perifere pressor effecten. Door dieren kort na de geboorte vasopressine toe te dienen kan de werking van vasopressinerge systemen op volwassen leeftijd veranderd worden. De dieren werden op volwassen leeftijd getest in een geconditioneerde emotionele stress-situatie (hoofdstuk 6). Op deze manier worden de effecten van directe endocriene en perifere pressor van vasopressine toedieningen op het gedrag en autonome functies vermeden. Het blijkt dat in deze dieren de vasopressine toediening na de geboorte een sterke invloed heeft op het volwassen gedrag in een doolhof en op het passieve vermijdingsgedrag. De effecten van neonatale vasopressine toediening op de stress-geïnduceerde bradycardierespons zijn veel minder sterk. Deze resultaten wijzen op een direct centrale werking van vasopressine op gedrag, terwijl de manier waarop de bradycardie respons door vasopressine gemoduleerd wordt nog niet duidelijk is.

Om beter te kunnen begrijpen via welk mechanisme vasopressine gedrag en autonome responsen beïnvloedt, zijn in rust- en stress-situaties gedrag en neuroendocriene

variabelen gemeten in jong volwassen dieren. In rust blijkt vasopressine een sterke stijging te veroorzaken in bloedglucose en plasma corticosteron spiegels. Het plasma noradrenaline gehalte wordt sterk onderdrukt. In stress-situaties blijkt 1 uur na vasopressine toediening de sympathische noradrenaline respons nog steeds onderdrukt te worden. Vasopressine verhoogt tijdens rust het poetsgedrag. Verder wordt de stress-geïnduceerde gedragsactivatie in de met vasopressine behandelde dieren sneller onderdrukt, hetgeen weerspiegeld wordt in een snellere toename van het rustgedrag na de stress. De resultaten geven aan dat de onderdrukking van de stress-geïnduceerde noradrenerge activatie een belangrijke rol kan spelen in de versterkte bradycardie respons tijdens emotionele stress na vasopressine toediening en dat behalve een direct centraal nerveuze werking, perifeer toegediend vasopressine ook via neuroendocriene en bloedglucose responsen een modulerende werking op het gedrag kan hebben.

Samenvattend kan worden geconcludeerd dat de parasympathische reactiviteit algemeen verminderd is in oude ratten, waarbij waarschijnlijk aminerge systemen in de limbische structuren van het centrale zenuwstelsel een belangrijke rol spelen. Vasopressine kan het gedrag en autonome responsen moduleren op meerdere centraal nerveuze en perifere werkingsniveaus. Oude dieren vanaf 20 maanden zijn echter ongevoelig voor deze modulaties door perifeer toegediende vasopressine. De verminderde parasympathische reactiviteit lijkt in oude dieren niet noodzakelijk te leiden tot een verstoring van de autonome balans omdat ook de sympathische reactiviteit afgenomen is. Er bestaan echter risicofactoren, bijvoorbeeld vetzucht en chronische stress, die een dergelijk evenwicht in fysiologische functies tijdens veroudering bedreigen. Met een sterke toename van het aantal ouderen in onze samenleving, zal de studie naar de invloed van deze risicofactoren op het verouderingsproces steeds dringender worden.

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List of Publications

Full Papers

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Abstracts

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